



## Manual to determine gonadal maturity of Baltic herring

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**Manual to determine gonadal maturity of herring (*Clupea harengus* L.)**

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# ***Preface***

This manual was developed with the purpose to improve the quality of maturity data of herring (*Clupea harengus* L.), and thereby the basis for estimation of spawning stock biomass applied in stock assessment and management as an indicator of stock reproductive potential. The maturity scale presented includes eight maturity stages for each sex. The macroscopic maturity stages are based on a histological evaluation of gonadal development. The manual and analyses were based on samplings of herring obtained from the central and western Baltic.

The macroscopic stages are illustrated in different ways in order to reduce differences in subjective judgement and thereby increase the accuracy of stage identification using the approach of Tomkiewicz et al. (2002). In addition, light microscopy of oocytes is suggested as a means of immediate verification, while histological criteria are included as a means for subsequent validation. The histological classification criteria are included and similarly illustrated.

The manual will provide the basis for an improvement of the quality of the data collected according to the EU fisheries data collection regulation (DCR). Furthermore, the manual will be an important tool for ongoing work by ICES PGCCDBS (Planning Group for Commercial Catches Discard and Biological Sampling) on standardisation and quality assurance of fisheries data collection. Sampling, analyses and the elaboration of the manual was primarily supported by DTU Aqua.

Sampling was conducted during the Baltic International Trawl Survey (BITS) and we acknowledge the following people at DTU Aqua, who participated in the sampling, histological analyses, and/or provided constructive feedback on previous versions of the manual: Kirstine Underbjerg Larsen, Inger Hornum, Lotte Worsøe Clausen, Stina B. Stenersen Hansen, Susanne Hansen and Henrik Mosegaard. We appreciate the collaboration with Gerd Kraus (*Johann Heinrich von Thünen-Institut*, Federal Research Institute for Rural Areas, Forestry and Fisheries, Hamburg, Germany), who helped us obtaining spawning herring. We thank Edward A. Trippel (St. Andrews Biological Station, St. Andrews, Canada), Olav Kjesbu (Institute of Marine Research, Bergen, Norway) and Gavin Power (Galway-Mayo Institute of Technology, Galway, Ireland) for a useful dialogue about characteristics of the maturity stages and suggestions for improvement of the manual.

Technical University of Denmark  
National Institute of Aquatic Resources  
Charlottenlund, October 2008

# ***Introduction***

## **Herring populations**

Herring commonly migrate between feeding areas and spawning grounds every year. Baltic herring typically spawn in bights or estuaries on shallow water banks (Rajasilta *et al.* 1993), while other herring populations seek deeper water banks (Runnström 1941, Parrish & Saville 1965). Herring are divided into stocks, populations and races according to morphological characteristics and where and when they spawn (ICES 2003). Herring populations mix with each other during spawning or feeding seasons and individuals belonging to different populations can often only be distinguished by meristic characters such as otolith microstructure and shape or genetic analyses (Bekkevold *et al.* 2005, Podolska 2006, Clausen *et al.* 2007).

Herring sampled in the Baltic Sea have been used as examples in this manual. In the central Baltic Sea, herring spawning in spring (March-April) dominates, but herring spawning in autumn (September-October) are also present. In the western Baltic Sea, the spring-spawning Rügen herring migrates to feeding areas as far away as the North Sea, but also local residing populations exist (Aro 1989, Bekkevold *et al.* 2005, Clausen *et al.* 2007).

## **Reproductive biology**

Herring is iteroparous with determinate fecundity and group-synchronous oocyte development (Murua and Saborido-Rey 2003). In other words, herring can spawn several times during a lifetime, the amount of eggs to be spawned in a given year is determined early in the maturation phase, and the recruited oocytes develop synchronously. All eggs are spawned during one spawning event covering a relatively short time period. The eggs are very sticky and adhere to the substrate, which in the Baltic Sea may be filamentous algae or aquatic plants such as pondweed (Rajasilta *et al.* 1993), and in the North Sea more commonly small stones or gravel (Parrish & Saville 1965). The eggs are fertilised by the male herring swimming close to the female and releasing milt over the eggs (Holliday 1958).

## **Sampling of gonads and fresh mounts**

Gonads used to develop the maturity scale were sampled onboard two research cruises in the Bornholm Basin of the Baltic Sea during November 2004 and March 2006, and from commercial catches in Kiel Bight in May 2006. Samples from different times of the year provided gonads in different developmental stages of the reproductive cycle. The sampling considered fish length in order to cover different sizes of herring within maturity stages. Of each sampled fish, length and body weight were recorded and the gonad was weighed and photographed together with the fish prior to preservation in a 4% buffered formaldehyde solution.

From a subset of samples, fresh mounts of ovarian tissue from females in different developmental stages were photographed to illustrate the characteristics of un-preserved oocytes. Kjesbu (1991) applied a similar approach photographing fixed oocytes in whole-mount preparations of cod ovarian tissue. In the present analysis, samples from the same ovaries were preserved and analysed histologically for verification of oocyte development.

### Histological analysis and classification

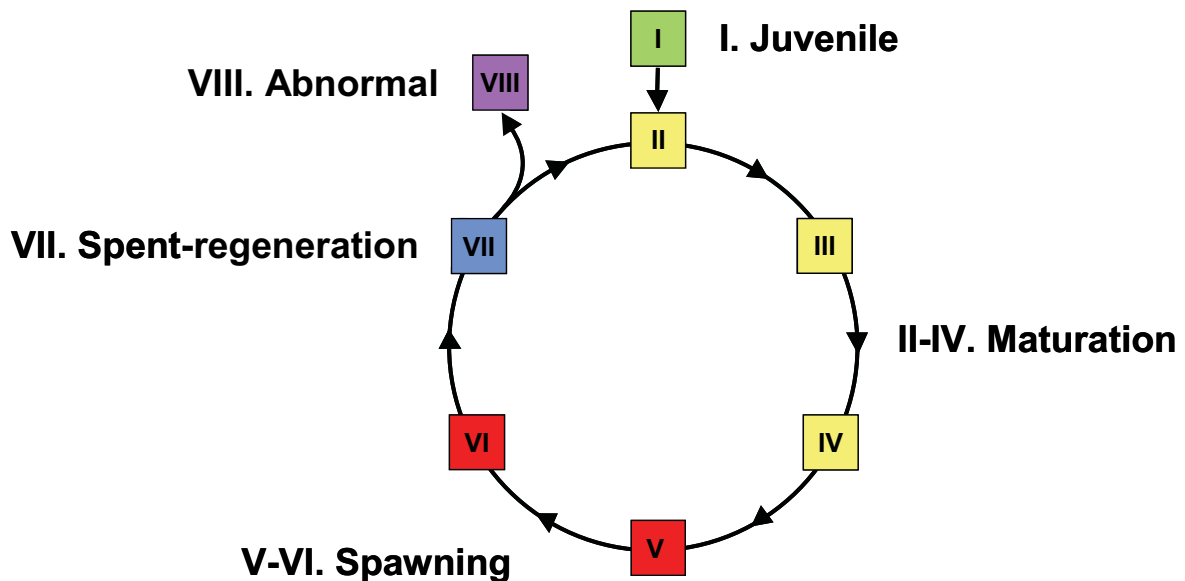
The sampled ovaries and testes were analysed histologically in the laboratory. A section of the middle part of one gonad lobe was selected and embedded in paraffin, sectioned at 7 µm and stained using H&E.

Gonadal development is a continuous process, but specific histological characteristics can be used to classify stages of gonadal development during the reproductive cycle. The histological analyses of reproductive status considered previous work on herring by Bowers and Holliday (1961) as well as a guide to macroscopic and microscopic maturity stages of Atlantic herring by Landry and McQuinn (1988).

The microscopic criteria applied in the classification of ovarian development are based on oocyte characteristics such as the formation of cortical alveoli, degree of yolk accumulation and nuclear migration. For males, criteria such as the presence/absence and relative proportion of spermatogonia, spermatocytes and spermatozoa were applied. The criteria applied to classify maturity histologically are given in the illustrated guide to histological classes on pages 10 and 11.

### Reproductive cycle and maturity stages

The developed and validated maturity scale includes eight stages per sex, which are described on pages 12 to 13. The maturity stages can be grouped into five phases: I. Juvenile; II.-IV. Maturation; V.-VI. Spawning; VII. Spent-regeneration; and VIII. Abnormal. The figure below illustrates the stages and phases in relation to the reproductive cycle:



**Figure 1. Developmental stages and phases defined histologically and macroscopically from ovaries and testes of herring sampled in the Baltic Sea.**

The description of macroscopic criteria was developed by comparing the histological results with the photographic records of the gonads. The macroscopic stages are described and illustrated on pages 14 to 29 and 30 to 45 for females and males, respectively. For females, the development is further illustrated using light microscopy photos of fresh mounts. In the unpreserved oocytes, it is possible to identify e.g. the presence of cortical alveoli and accumulated yolk granules. This method can be applied at

sea using a light microscope for a rapid evaluation to distinguish whether a female is immature, in spent-regeneration stage or -maturing, and to ascertain that vitellogenesis has begun as suggested by Kjesbu (1991).

## Manual design

### *Histological maturity classes*

The histological classes are described for females and males on two separate pages. Only class I to VII are included in this description. For each class, information is given about the composition of cell types in the specific class and for females emphasizing the developing group of oocytes. Each class is illustrated by two photographs. The image to the left of the text is an overview of a larger area of the gonad section, while the image to the right is a close-up. For both males and females the overview photograph illustrates the different reproductive cell types present in the maturity class and the overall structure of the reproductive tissue. The characteristic oocytes for the specific female maturity class are shown in the close-up image, while for males it is simply a closer view of the composition of gamete types present in the specific maturity class.

### *Macroscopic maturity stages*

The description of stages provides information about the extension of the gonad in relation to the body cavity as well as the width of the gonad at the widest point. Relative size, shape, consistency and structure are the most important characteristics distinguishing maturity stages. Differences in colour between maturity stages that occur during development due to yolk and sperm formation are also described as well as the hydration of oocytes. The colour is a more prominent trait in females than males, as the variation in colour between testes in different maturity stages is more subtle than for ovaries. The most significant macroscopic characteristics of ovaries are the visibility and opaqueness of the oocytes, while for males it is the volume of the testes compared to the body cavity as well as the appearance of the sperm duct and milt. The male reproductive cycle in general exhibit a smoother transition between stages than the female cycle.

### *Illustrations*

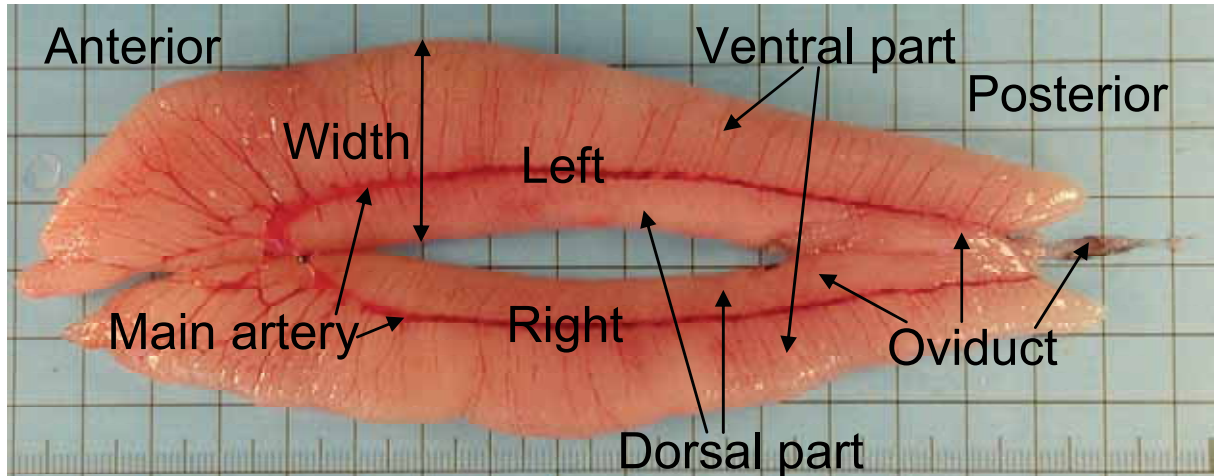
Each maturity stage is illustrated by two examples indicating the variation in appearance within the specific stage. In most cases, the first example is a specimen early in the specific maturity stage and the second later in the stage. The exceptions include stages II and III for females and stage II for males, where the first example is a juvenile undergoing its first maturation and the second example a repeat spawner in maturation. The first example is therefore often a smaller specimen than used in example two.

Each stage includes at least one photograph illustrating the appearance of gonads in the body cavity (except male stage II), a photograph of the gonad next to the fish and one or more close-ups of gonad structure or tissue. For each female stage a light microscopy photograph additionally illustrates the developmental characteristics of oocytes in fresh gonad tissue. **The squares on the blue background used in the photographs measure 1x1 cm.**

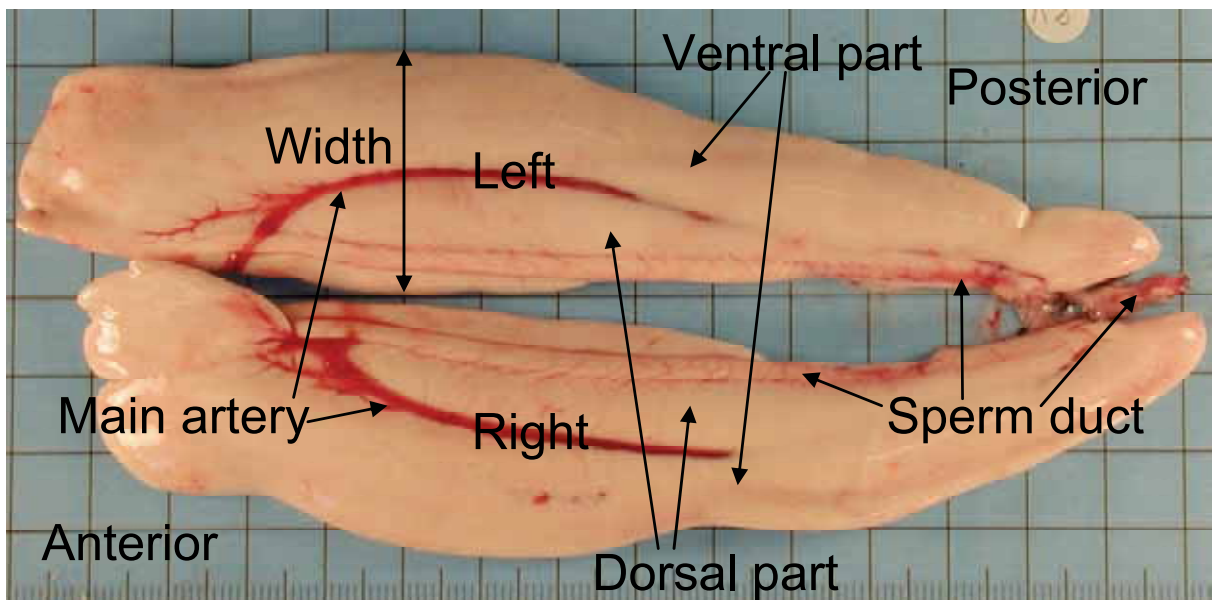
Figures 2 and 3 illustrate descriptors used to explain the position and morphological structures within ovaries and testes, respectively. The description dorsal and ventral parts of the lobes refer to their position in the body cavity of a fish. The lobes unfold when the gonad is removed from the body cavity, which makes macroscopic characteristics easier detectable. The left lobe (viewed from anterior and the ventral side of the fish) is normally the larger of the two.

### *Text boxes and close-ups*

Two text boxes accompany each example. The larger text box highlights important characteristics and provides tips for easy recognition of the stage in question. These characteristics are illustrated by close-ups of the gonad and additionally for females, light microscopy photographs of fresh mounts of ovarian tissue. The smaller text box shows information about the illustrated specimen including total length ( $L_T$ ), total body mass ( $M_B$ ), gonad weight ( $M_G$ ), gonadosomatic index ( $GSI = M_G / M_B * 100$ ), the sampling month and year (M) and the fish identification number (ID).



**Figure 2.** Descriptors used in the manual to explain the position of morphological structures, such as the oviduct. The location of the main artery is also depicted. Dorsal and ventral part refers to the position of the lobes in the body cavity of the fish.



**Figure 3.** Descriptors used in the manual to explain the position of morphological structures within herring testes, such as the spermatoduct. The location of the main artery is also depicted. Dorsal and ventral part refers to the position of the lobes in the body cavity of the fish.

## **Application of the manual**

### *Limitations*

This manual utilises herring in the Baltic Sea. Therefore, the manual may not fully cover visual and meristic characteristics of herring populations in other areas. In particular, the colour of ovaries can vary among stocks. The width span of gonads in different maturity stages should be considered on a relative scale for other stocks, since herring races vary considerably in size. Maturity stages are sometimes determined from frozen samples and in this case the characteristic traits provided for stages may be difficult to discern.

A resting stage or skip of spawning stage is not included separately in the maturity scale. Such a stage may exist and prevail in other areas and in some periods, but it did not occur in the specimens obtained in the present samplings. A gradual transition between spent, recovering, and rematuring testes was observed for males, but not for ovaries.

### *Lower size limits for sex and maturity determination*

The gonads of herring are relatively long and easily detectable even in very small and immature individuals. The characteristic transverse grooves along the ventral side of the cylindrical ovaries, which are due to internal septa, compared to the even structure of the blade shaped testes makes the macroscopic distinction between the two sexes relatively easy. During the three sampling events, no fish under the length of 11 cm was obtained and the sex and maturity stage of all specimens could be determined. It seems realistic that the sex of even smaller individuals can be judged macroscopically.

### *Maturity data for stock assessment*

The optimal sampling time to estimate the proportion of spawners and hence herring spawning stock size is around 1-2 months before the start of the spawning season. For spring spawners in the Baltic Sea, the optimal sampling time is in February-March, while for autumn spawners it is July-August. However, establishment of traditional stock specific maturity ogives for herring e.g. in the Baltic Sea is hampered by the mixing of spring and autumn spawners in the surveyed areas, because immature specimens cannot be designated to a specific stock by visual judgement.

Within seasons, spawners however can often be separated into spring spawners and autumn spawners by the degree of maturation. Stage III-VI specimens caught in January-April will be spring spawners, while stage VII and II specimens will be autumn spawners. Likewise stage III-VI specimens caught in July-September will be autumn spawners, while stage VII-II specimens will be spring spawners. During October-December and May-June, the proportion of the stock that will participate in the following spawning season cannot accurately be determined. At these times, spent individuals (stage VII) and rematuring individuals (stage II) are difficult to separate visually. These might be distinguished by checking the presence/absence of cortical alveoli or yolk granules by light microscopy in females, however, the determination will be time consuming and imprecise due also to the presence in the stock of less developed first-time spawners.

We recommend using a spawning probability function that expresses the proportion of spawners i.e. proportion maturing specimens in the total population in spring and in autumn respectively to obtain more precise estimates of spawning stock size per stock. The spawning probability function thus defines the proportion per age or length group that can be assumed to spawn in the following spawning season. If the proportion of spawners is determined 1-2 months before the spawning season, the spawners will include specimens in stages III, IV, V and potentially VI, while the proportion of non-spawners includes specimens in stages I, II, VII and VIII.



### Comparison with other herring maturity scales

A scale, which was recommended by the ICES Herring Committee in 1963, is used in some laboratories. Bowers and Holliday (1961), Landry and McQuinn (1988), Parrish & Saville (1963) and ICES 1963 scale are similar and all include a recovering-spent stage (VIII) which encompasses the final recovery of the spent gonad as well as the beginning of a new maturation cycle. We have split this stage in two, leaving the late recovering in stage VII and the rematuring in stage II. Furthermore, we have included a stage covering reproductive malfunction (Stage VIII). Table 1 describes the conversion of the scale used in this manual to the ICES (1963) scale as well as the scales used for the BITS and IBTS surveys.

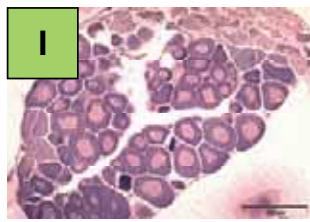
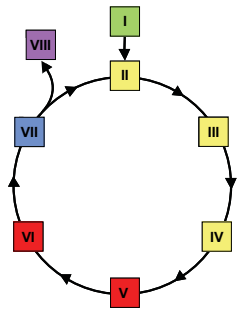
**Table 1. Conversion of the maturity scales currently in use.**

Present scale	ICES 1963	BITS	IBTS
I. Juvenile	I. Virgin	I. Virgin	I. Immature
II. Early maturation	II. Virgin maturing VII. Recovering-spent	II. Maturing	II. Maturing
III. Mid maturation	III. Maturing		
IV. Late maturation	IV. Maturing		
V. Spawning capable	V. Maturing		
VI. Spawning	VI. Spawning	III. Spawning	III. Spawning
VII. Spent-recovery	VII. Spent	IV. Spent V. Resting	IV. Spent
VIII. Abnormal			

### References

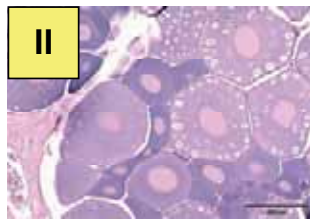
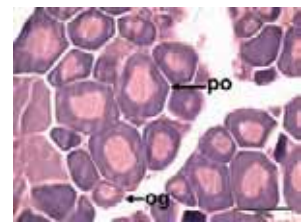
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# Histological classes ♀



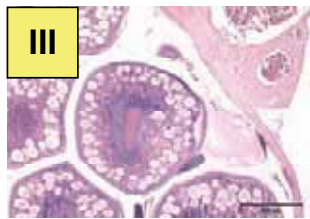
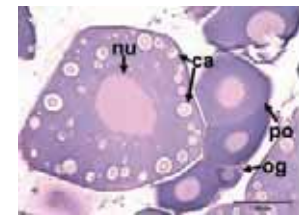
## Juvenile

Only oogonia (og) and primary growth oocytes (po) with large pink nuclei are present. Ovary wall is thin. Atresia may occur in the present and all of the following classes. Scale bar (Sb): 100 µm.



## Early maturation

Secondary growth oocytes in the cortical alveoli (ca) stage, primary growth oocytes and oogonia are present. Ovary wall is thin in first time spawners, thick in repeat spawners. The ca oocytes are enlarged and the ratio nucleus (nu) to oocyte area has decreased. Sb: 100 µm.



## Mid maturation

Secondary growth oocytes containing yolk granules (yg), primary growth oocytes and oogonia are present. The oocyte diameter increases dramatically during III. By the end of class III the cytoplasm is half filled with yolk granules. Sb: 100 µm.



## Final maturation

Secondary growth oocytes in the nuclear migration stage, primary growth oocytes and oogonia are present. By end of class IV oocytes are completely filled with yolk, cortical alveoli are pressed against cell membrane (cm) and nucleus begins migration towards micropyle. Sb: 200 µm.



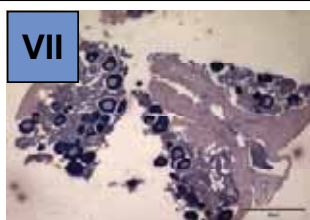
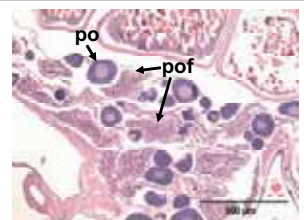
## Spawning prepared

Final growth oocytes with hydrolyzed yolk granules (hyg), primary growth oocytes and oogonia are present. The nucleus has disintegrated and is no longer visible. Sb: 200 µm.



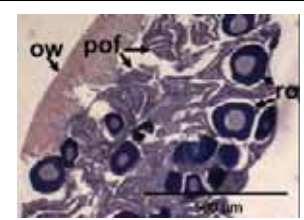
## Spawning active

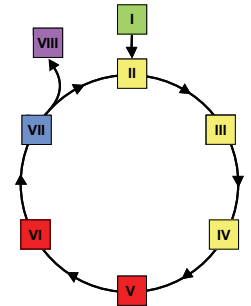
Post ovulatory follicles (pof), final growth oocytes with hydrolyzed yolk granules, primary growth oocytes (po) and oogonia are present. Ovulated eggs are present in the dorsally located oviduct. POFs accumulate in the peripheral tissue. Sb: 500 µm.



## Spent-regeneration

Primary growth oocytes and oogonia are present. Post spawning: Numerous POFs. Regeneration: Numerous primary growth oocytes, no POFs. The ovary wall (ow) is thick from the contraction of the ovary after spawning. Sb: 500 µm.





	<p><b>Juvenile</b> A mix of primary germ cells and spermatogonia are present. The tubulate structure is not developed in this class. More spermatogonia than primary germ cells are present by end of class. Scale bars (Sb): Left 100 <math>\mu</math>m, right 50 <math>\mu</math>m.</p>	
	<p><b>Early maturation</b> Spermatogonia (sg), primary (ps) and secondary spermatocytes (ss), and spermatids (st) are present. Tubular walls (tw) are developed. Appearance of spermatozoa in the center of some of the tubules marks the end of class II. Sb: Left 500 <math>\mu</math>m, right 50 <math>\mu</math>m.</p>	
	<p><b>Mid maturation</b> Spermatozoa (sz) are present in the center of most of the tubules. Spermatogonia, primary and secondary spermatocytes, and spermatids are also visible. By the end of class III spermatozoa occupy 1/3 of tubule area. Sb: Left 500 <math>\mu</math>m, right 50 <math>\mu</math>m.</p>	
	<p><b>Final maturation</b> Spermatogonia production ends. Finally only a narrow band of spermatocytes can be seen. Primary germ cells and spermatogonia prevail near the tubule wall. Spermatozoa and few spermatids occupy up to 3/5 of tubule area. Sb: Left 500 <math>\mu</math>m, right 50 <math>\mu</math>m.</p>	
	<p><b>Spawning prepared</b> Spermatozoa completely occupy the tubules late in class V, with exception of resting primary germ cells and spermatogonia. Spermatozoa are characteristically attached by the head to Sertoli cells and flagella are easily visible. Sb: Left 500 <math>\mu</math>m, right 50 <math>\mu</math>m.</p>	
	<p><b>Spawning active</b> The spermatozoa are now uniformly distributed in the tubules and flagella are difficult to distinguish. The spermatozoa are detached from the tubule wall and tubules are progressively emptied of sperm. Sb: Left 500 <math>\mu</math>m, right 50 <math>\mu</math>m.</p>	
	<p><b>Spent-regeneration</b> Residual sperm may be present in some tubules. A new line of germ cells are produced and gradually testes enter class II. Testicular and tubule walls are thick and valved from contraction of testes during spawning. Sb: Left 500 <math>\mu</math>m, right 200 <math>\mu</math>m.</p>	



## Maturity scale ♀

Stage	Macroscopic characteristics to determine gonadal maturity of females
<b>I. Juvenile</b>	Ovaries emerge as thin, flat paired organs occupying around 1/2 - 2/3 of the length of the body cavity; 1-1½ mm wide; transparent, but may range from transparent to yellow-orange translucent; distinguishable from testes by thicker appearance, and grooved surface due to septa within the gonad; oocytes not visible to the naked eye.
<b>II. Early maturation</b>	First time spawners: Ovaries occupy 1/2 to 2/3 or more of the length of the body cavity; 3-5 mm wide, slightly narrower at posterior end; transparent yellow to orange; shape more cylindrical; grooves less pronounced; single oocytes can not be detected with the naked eye. Repeat spawners: Ovaries occupy 1/2 to over 2/3 of the length of the body cavity; 4-10 mm wide, slightly narrower at the posterior end; colour varies from translucent orange with red tints to wine-red often with transparent or orange appearance anterior and posterior; cylindrical shape; single oocytes are not visible.
<b>III. Mid maturation</b>	Ovaries occupy between 2/3 and the entire length of the body cavity; 5-12 mm wide and narrower towards the posterior end; colour varies from early stage translucent orange with red tints to late stage opaque red-orange or yellow-pale; oocytes easily detectable with naked eye. In this and later stages maturation it is more difficult to distinguish between first time and repeat spawners. But a more reddish colour is often due to a thickened tunica indicating previous spawning.
<b>IV. Final maturation</b>	The ovaries occupy the entire length of the body cavity; around 10-20 mm at the widest point and narrower towards the posterior end; colour is opaque and varies from orange to pale yellow or almost whitish; oocyte diameter has increased. Arteries within the tunica of the ovary disrupt easily when handled and red blotches may occur. The texture of the ovary is very "crisp" and it therefore also breaks easily.
<b>V. Spawning prepared</b>	Ovaries occupy the entire volume of the body cavity; 20-30 mm at the widest point and narrower towards the posterior end; colour is translucent whitish to mother of pearl in late stage; no eggs are released with slight pressure to the abdomen. Specimens are close to spawning, but the most important traits of this stage are: 1) Large volume of the ovary 2) Transparency of the eggs 3) No eggs released with slight pressure to the abdomen.
<b>VI. Spawning active</b>	Ovaries still occupy the full length of the body cavity, but the ventral part gradually shrinks during spawning; colour is bright translucent yellow; eggs are released with slight pressure to abdomen or flow freely; ovary consistency is soft and surface smooth. The ventral half of the ovary becomes increasingly striated and bloodshot, as the eggs are gradually ovulated and released into the dorsally located oviduct.
<b>VII. Spent - regeneration</b>	Ovaries occupy from less than 1/2 to more than 2/3 the length of the body cavity; 5-8 mm wide, but narrower towards the posterior; translucent orange to red; some with clear transparent posterior and/or anterior ends; flaccid and shrunk; main artery prominent. As the ovary wall contracts the transverse grooves appear again and the ovary becomes more firm. As in stage II repeat spawners the transverse grooves sometimes appear grey.
<b>VIII. Abnormal</b>	Ovaries are subject to a variety of developmental abnormalities. Although abnormalities occur relatively infrequently it is important to recognize such phenomena as potential indicators of ecosystem changes such as increased amounts of pollutants.

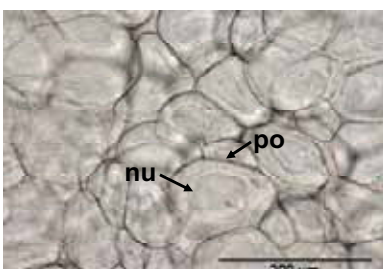
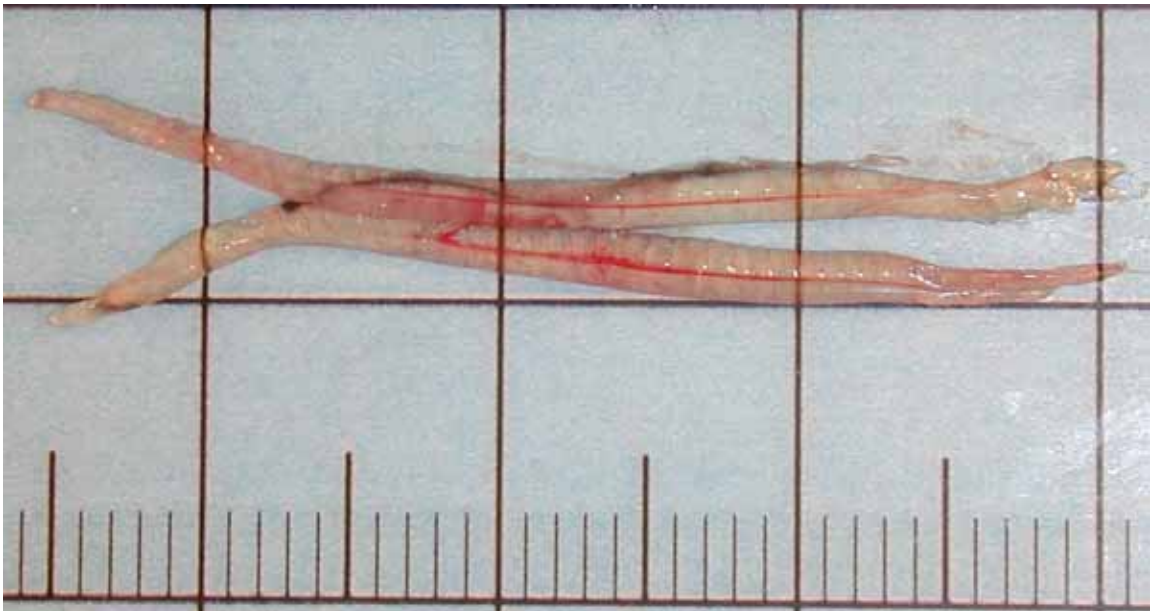
Stage	Macroscopic characteristics to determine gonadal maturity of males
<b>I. Juvenile</b>	Testes emerge as thin, flat paired organs occupying around 1/3 – 1/2 of the length of the body cavity; a few mm wide; transparent to whitish translucent; distinguishable from ovaries by the lanceolate shape; the sperm duct is not visible to the naked eye.
<b>II. Early maturation</b>	First time spawner: Testes occupy 1/2 the length of the body cavity; around 5 mm wide, but narrower towards the posterior; translucent whitish-pink; edges remain transparent; the sperm duct is visible early in the stage as a tubule dorsally. Repeat spawner: Testes occupy 2/3 or more of the length of the body cavity; 5-15 mm wide, narrower towards the posterior; translucent pink to orange-red; edges transparent; shape clearly lanceolate; sperm duct is visible as a tubule running almost the entire length dorsally.
<b>III. Mid maturation</b>	Testes occupy between 2/3 and the entire length of the body cavity; 5-15 mm wide and narrower towards the posterior end; colour varies from pale orange-pink to pale whitish-pink; increasingly opaque but transparent edges persist until late in stage. At this and later stages of maturation it is not possible to distinguish between first time and repeat spawners with the naked eye.
<b>IV. Final maturation</b>	The testes occupy the entire length and almost the entire volume of the body cavity; 15 - 25 mm at the widest point and narrower towards the posterior end; colour is opaque and very pale whitish-pink with white tints. Generally the colour varies little between different specimens in this stage.
<b>V. Spawning prepared</b>	Testes occupy the entire volume of the body cavity; 15 to more than 30 mm at the widest point and narrower towards the posterior; colour opaque, pale pinkish-white to milky-white; a small amount of sperm may be released with pressure, but sperm does not flow freely. Generally the colour varies only little between different specimens in this stage, but red tints may be visible in some tissues.
<b>VI. Spawning active</b>	Testes still occupy the full length of the body cavity, but width gradually diminish during spawning; colour opaque, pale pinkish-white to milky-white; testes consistency is soft and surface smooth; sperm flows freely. As spawning progresses the testes become increasingly striated and bloodshot, as sperm is released into the dorsally located sperm duct.
<b>VII. Spent - regeneration</b>	Testes occupy from around 1/2 to 2/3 the length of the body cavity; 3-8 mm wide, but narrower towards the posterior; translucent pale pink, orange or reddish-orange; transparent along edges; flat and shrunken; grooves running longitudinally; main artery prominent. By the end of stage VII spawning marks are less evident and in some cases it is only possible to distinguish stage VII and stage II repeat spawners histologically.
<b>VIII. Abnormal</b>	Testes are subject to a variety of developmental abnormalities. Although abnormalities occur relatively infrequently it is important to recognize such phenomena as potential indicators of ecosystem changes such as increased amounts of pollutants.

## I. Juvenile ♀

### I. Juvenile



Ovaries emerge as thin, flat paired organs occupying around 1/2 - 2/3 of the length of the body cavity; 1-1½ mm wide; transparent; distinguishable from testes by thicker appearance, and a grooved surface due to septa within the gonad; oocytes not visible to the naked eye.



#### Fresh sample

Translucent primary oocytes (po) of different sizes are seen. Cells are characterized by large nuclei (nu). Scale bar: 200 µm.

#### Specimen data

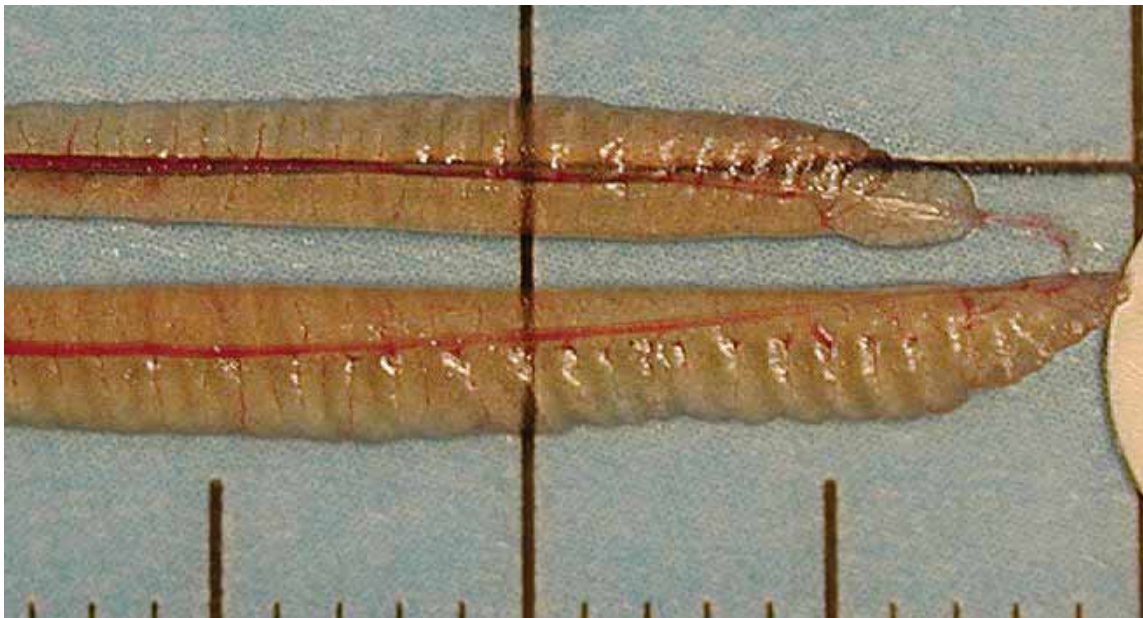
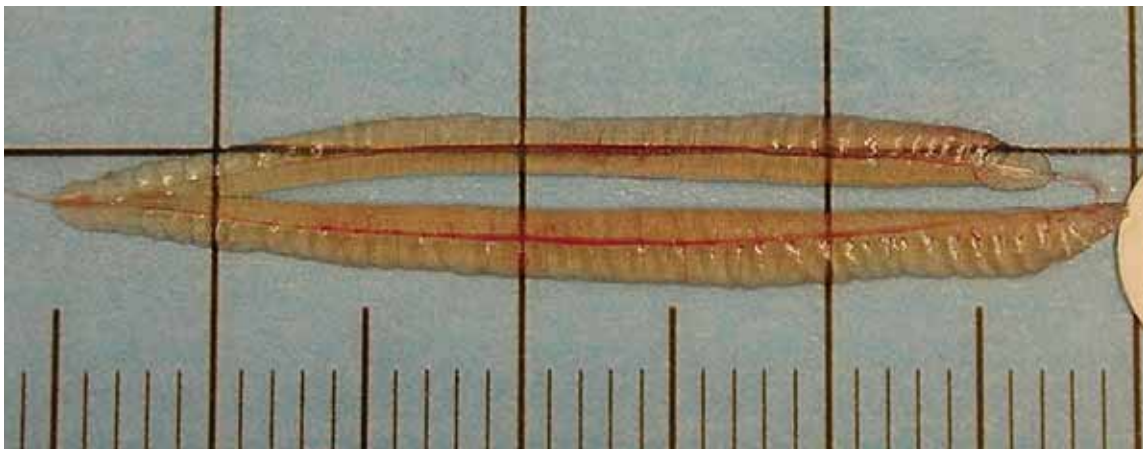
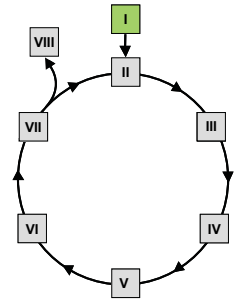
L<sub>T</sub>: 14.1 cm  
M<sub>B</sub>: 16 g  
M<sub>G</sub>: < 0.1 g  
GSI: ~ 0  
M: March 2006  
ID: 300306s/18



## I. Juvenile



This example illustrates the variation in colour and shape within stage I. The colour may range from transparent to yellow-orange translucent. The grooves on the ventral half of the ovary are easily detectable.



### Enlargement

No signs of previous spawning; the distal part of the oviduct is narrow.

**TIP:** It can be tricky to distinguish immature females from males. But if the gonad has transverse grooves on the ventral half it is a female!

### Specimen data

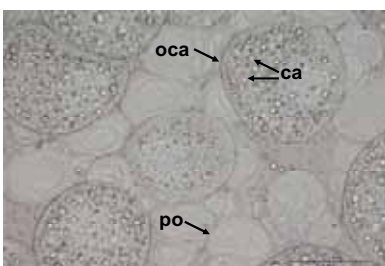
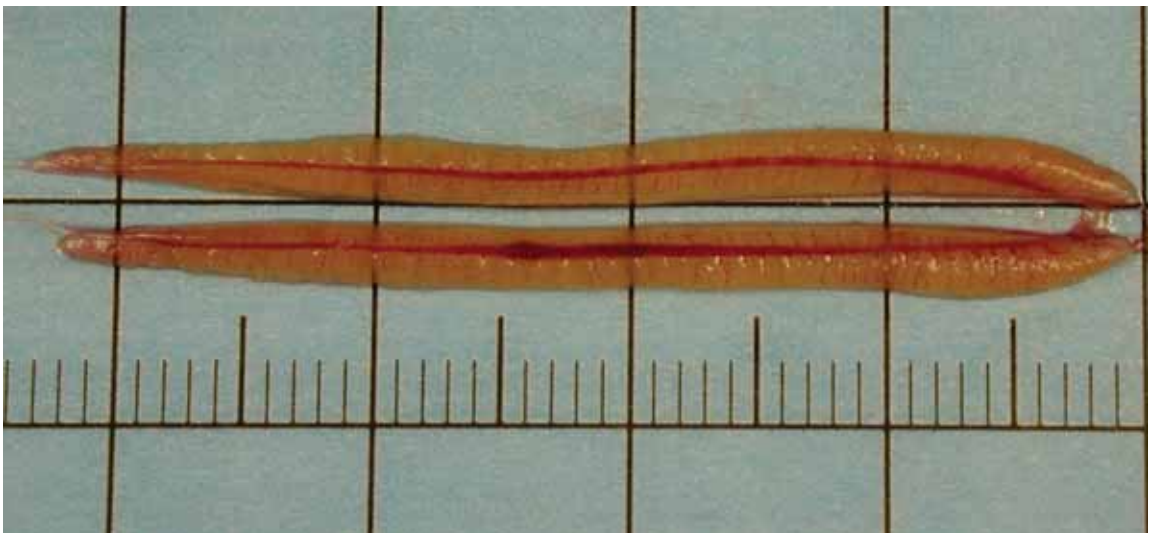
$L_T$ : 14 cm  
 $M_B$ : 17.3 g  
 $M_G$ : 0.1 g  
 $GSI$ : 0.6  
 M: November 2004  
 ID: 010405s/85

## II. Early maturation ♀

### II. Early maturation – first time spawner



Ovaries occupy 1/2 to 2/3 or more of the length of the body cavity; 3-5 mm wide, slightly narrower at posterior end; transparent yellow to orange; shape more cylindrical; grooves less pronounced; single oocytes can not be detected with the naked eye.



#### Fresh sample

Primary oocytes (po) and larger oocytes (oca) with cortical alveoli (ca) are present. Scale bar: 200  $\mu$ m.

**TIP: When ca are present the ovary has begun maturation. Ca can be verified by microscopy to separate stage II from stage I.**

#### Specimen data

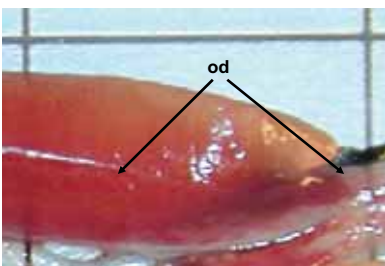
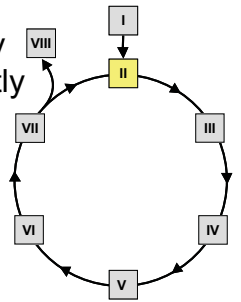
L<sub>T</sub>: 15 cm  
M<sub>B</sub>: 23.3 g  
M<sub>G</sub>: 0.2 g  
GSI: 0.9  
M: November 2004  
ID: 010405s/4



### II. Early maturation – repeat spawner



Ovaries occupy 1/2 to over 2/3 of the length of the body cavity; 4-10 mm wide, slightly narrower at the posterior end; colour varies from translucent orange with red tints to wine-red often with transparent or orange appearance anterior and posterior; cylindrical shape; single oocytes are not visible.



#### Enlargement

The oviduct (od) runs through the ovary dorsally and is enlarged towards the posterior as a sign of previous spawning.

**TIP:** It can be tricky to separate stage VII from stage II, but as for stage II first time spawners the presence of ca can be verified by microscopy.

#### Specimen data

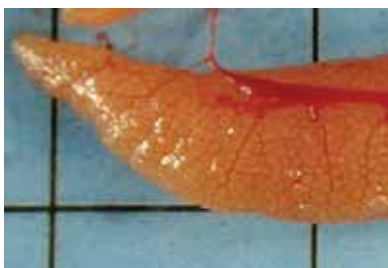
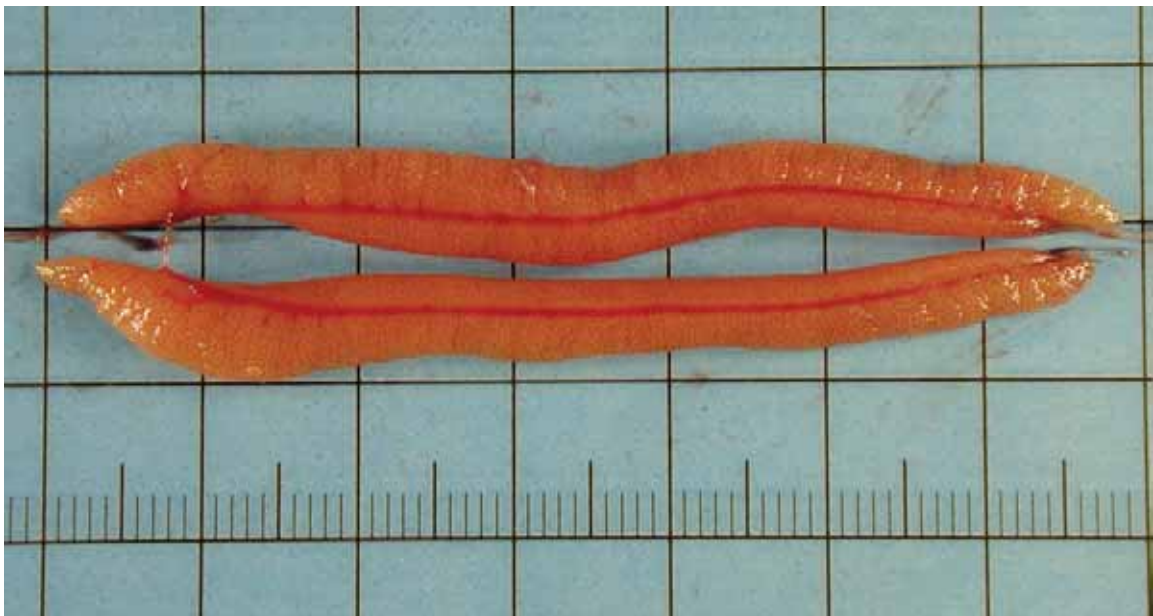
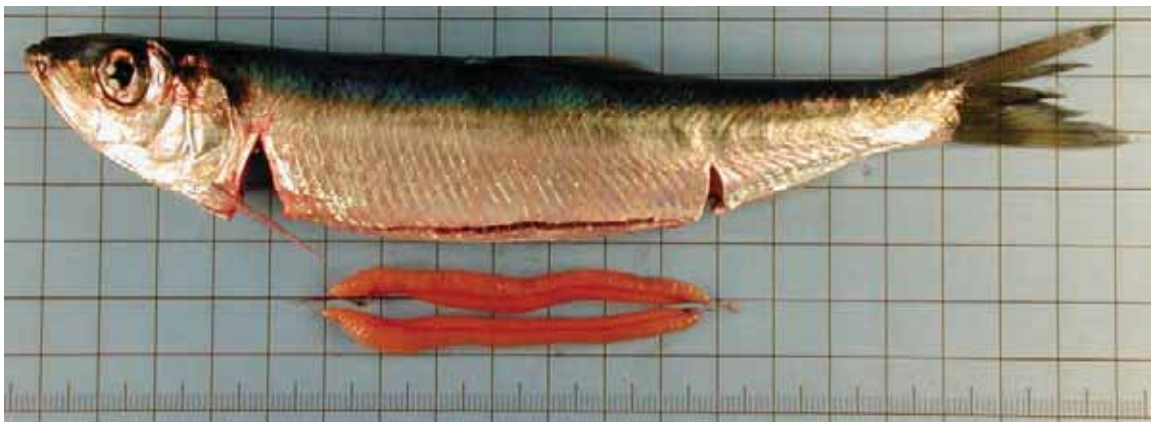
L<sub>T</sub>: 21.6 cm  
M<sub>B</sub>: 56 g  
M<sub>G</sub>: 1.1 g  
GSI: 2  
M: March 2006  
ID: 300306s/42

### III. Mid maturation ♀

#### III. Mid maturation



Ovaries occupy between 2/3 and the entire length of the body cavity; 5-12 mm wide and narrower towards the posterior end; colour varies from early stage translucent orange with red tints to late stage opaque red-orange or yellow-pale; oocytes easily detectable with naked eye.



#### Enlargement

The oocytes are now easily visible to the naked eye, and become increasingly opaque due to the incorporation of yolk granules.

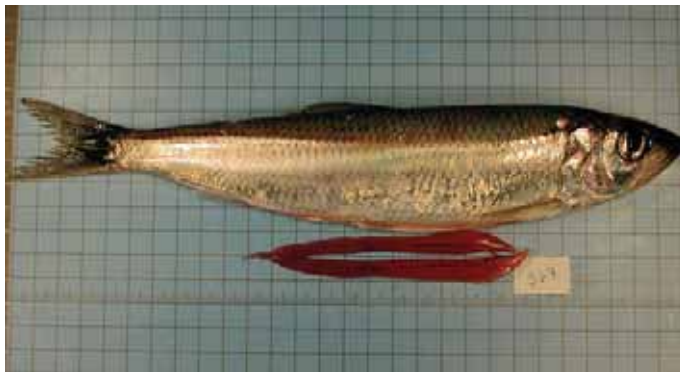
**Tip: The visibility of the oocytes is the most important criterion distinguishing between stages III and II.**

#### Specimen data

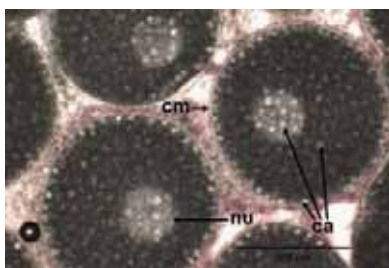
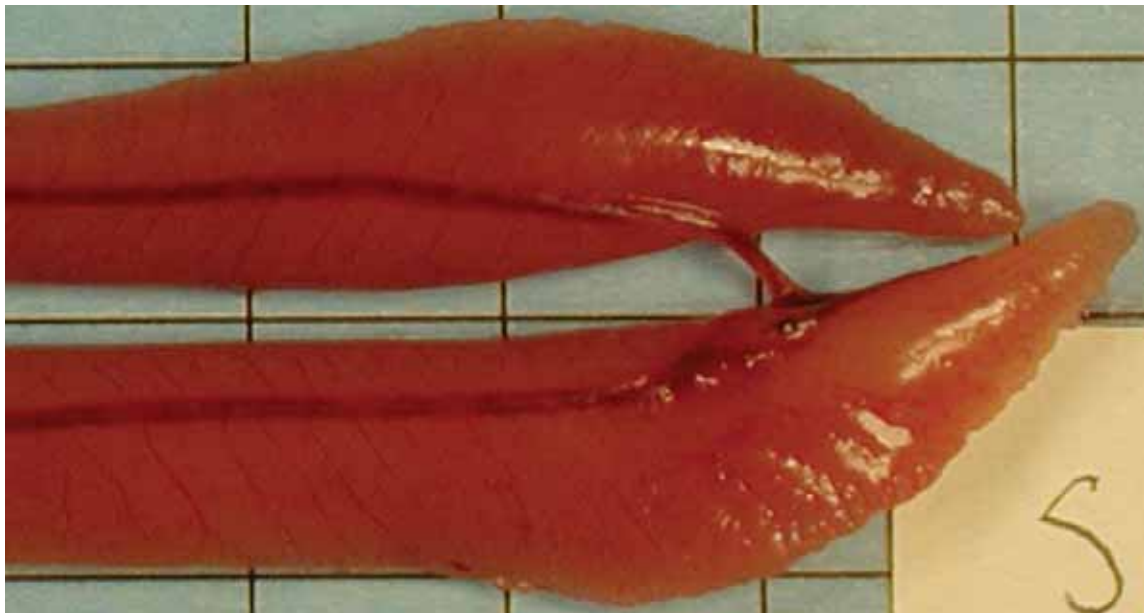
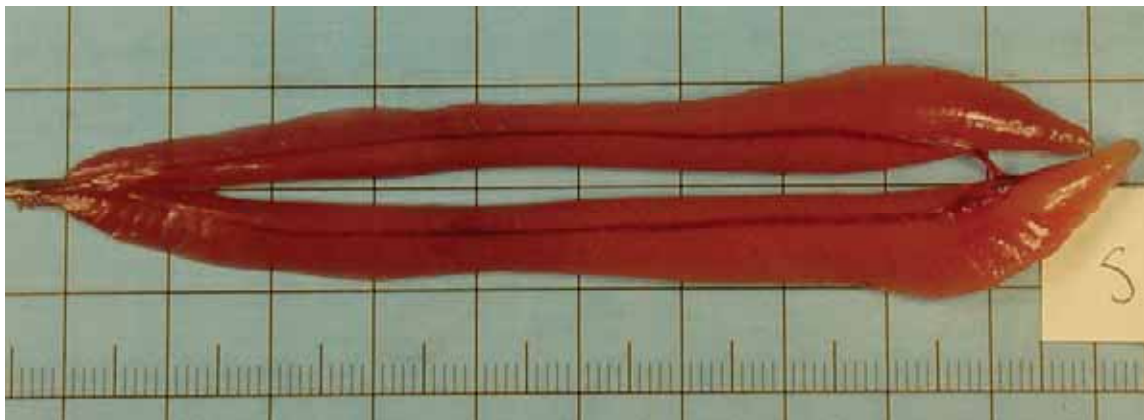
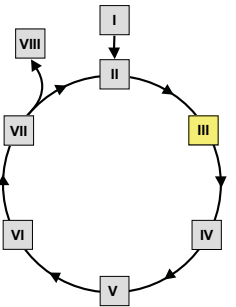
L<sub>T</sub>: 19.4 cm  
M<sub>B</sub>: 42 g  
M<sub>G</sub>: 1.6 g  
GSI: 4  
M: March 2006  
ID: 300306s/45



## III. Mid maturation



This example illustrates the variation in colour within stage III. In this and later stages of maturation it is more difficult to distinguish between first time and repeat spawners. But a more reddish colour is often due to a thickened tunica indicating previous spawning.



### Fresh sample

Oocytes with both cortical alveoli and yolk granules are present. Nucleus (nu) and individual cortical alveoli (ca) are visible, but not individual yolk granules. Oocytes are opaque due to yolk accumulation. The cell membrane (cm) is now visible. Scale bar: 500  $\mu$ m

### Specimen data

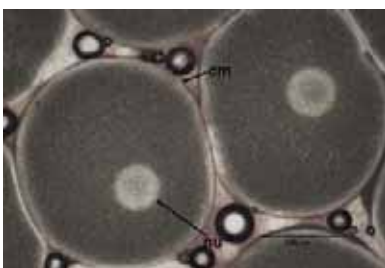
L<sub>T</sub>: 28 cm  
M<sub>B</sub>: 141.7 g  
M<sub>G</sub>: 4.6 g  
GSI: 3.4  
M: November 2006  
ID: 010405s/79

## IV. Final maturation ♀

### IV. Final maturation



The ovaries occupy the entire length of the body cavity; around 10-20 mm at the widest point and narrower towards the posterior end; colour is opaque and varies from orange to pale yellow or almost whitish; oocyte diameter has increased.



#### Fresh sample

Oocytes filled with yolk granules are present. Cortical alveoli are no longer visible, while the yolk granules can be detected. The nucleus (nu) to cytoplasm ratio has decreased. The width of the cell membrane (cm) has increased further. Scale bar: 500  $\mu$ m

#### Specimen data

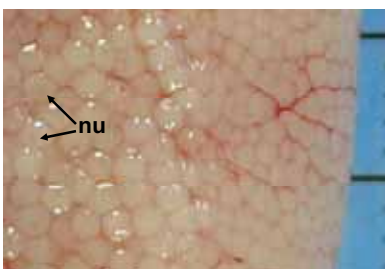
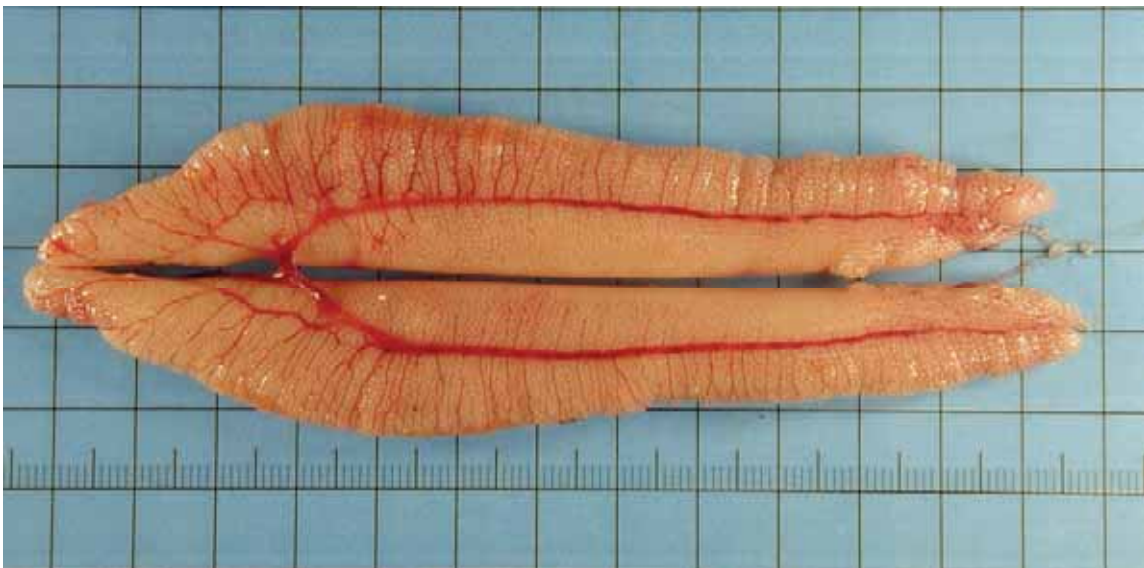
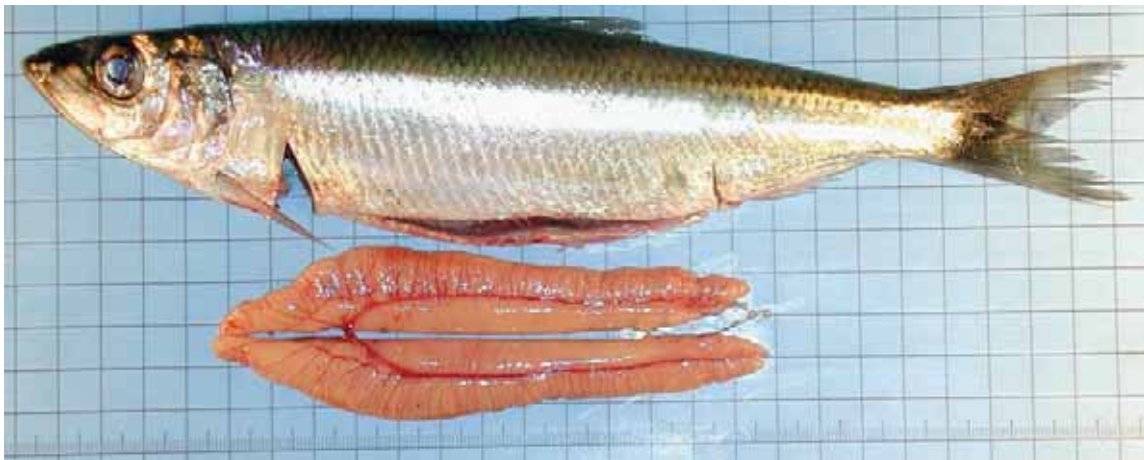
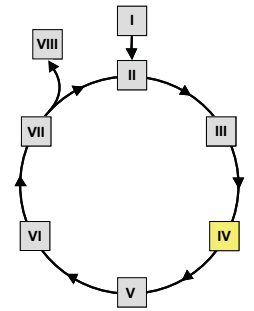
L<sub>T</sub>: 19.3 cm  
M<sub>B</sub>: 50 g  
M<sub>G</sub>: 5.4 g  
GSI: 12.1  
M: March 2006  
ID: 300306s/39



### IV. Final maturation



This example illustrates the variation in colour and size within stage IV. Arteries within the tunica of the ovary disrupt easily when handled and red blotches may occur. The texture of the ovary is very "crisp" and it therefore also breaks easily.



#### Enlargement

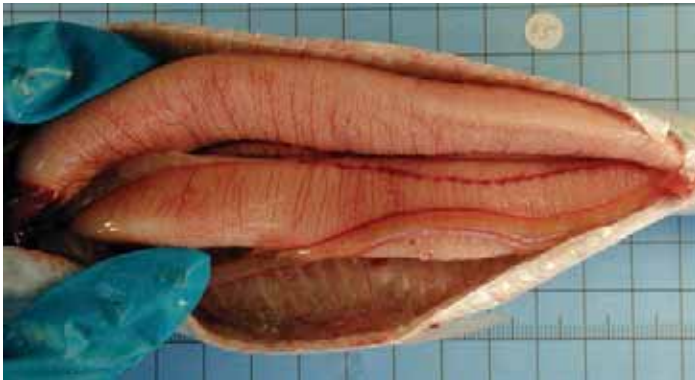
The most mature oocytes are now in the nuclear migration stage. The nucleus (nu) migrates towards the micropyle, through which the spermatozoa will enter and fertilize the egg.

#### *Specimen data*

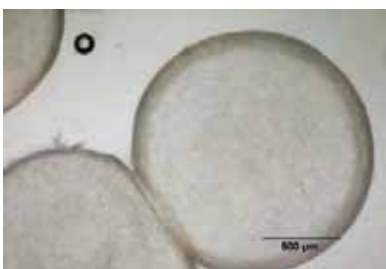
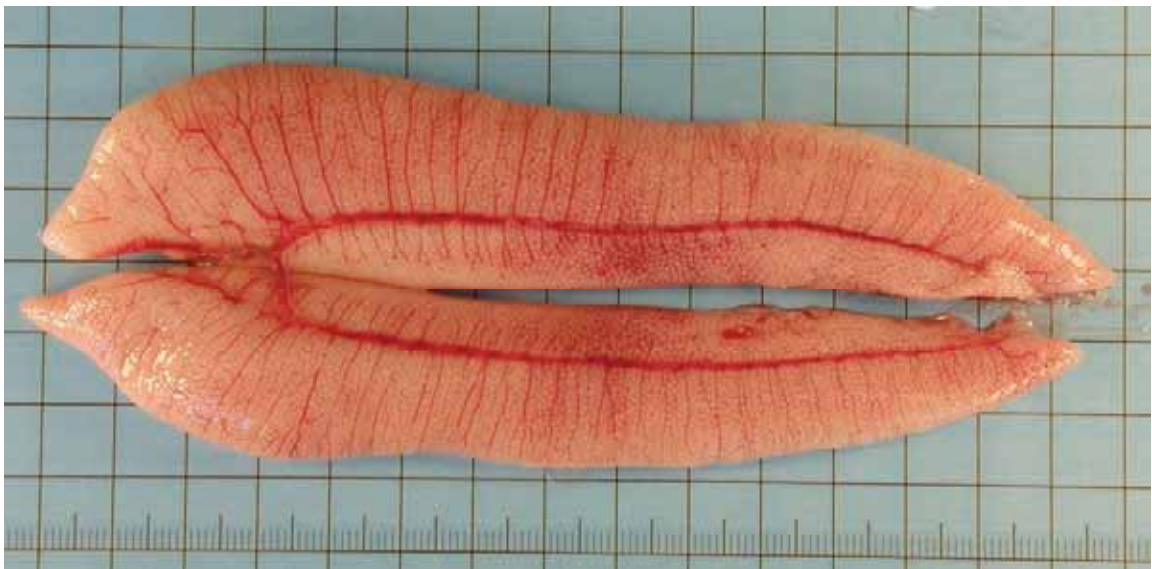
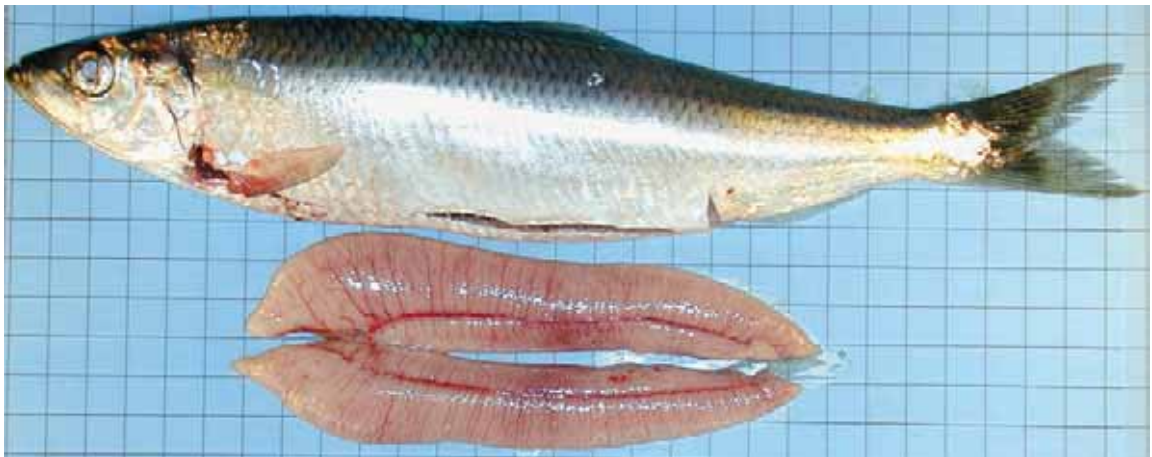
L<sub>T</sub>: 25.9 cm  
M<sub>B</sub>: 136 g  
M<sub>G</sub>: 25.8 g  
GSI: 25  
M: March 2006  
ID: 300306s/40

## V. Spawning prepared ♀

### V. Spawning prepared



Ovaries occupy the entire volume of the body cavity; 20-30 mm at the widest point and narrower towards the posterior end; colour is translucent whitish to mother of pearl in late stage; no eggs are released with slight pressure to the abdomen.



#### Fresh sample

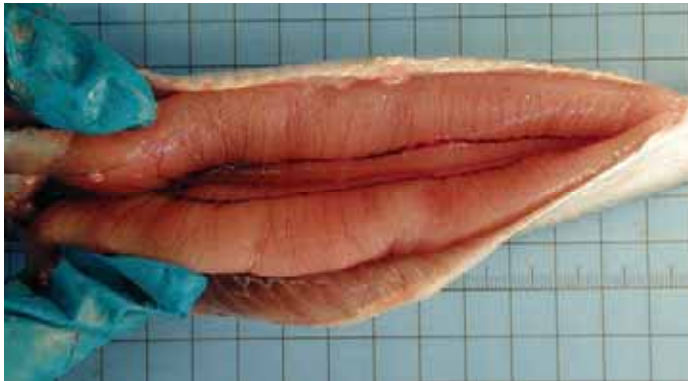
The yolk granules have now fused and hydrated. The eggs appear clear and translucent to transparent. Scale bar: 500 µm

#### Specimen data

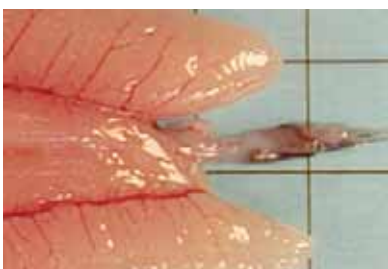
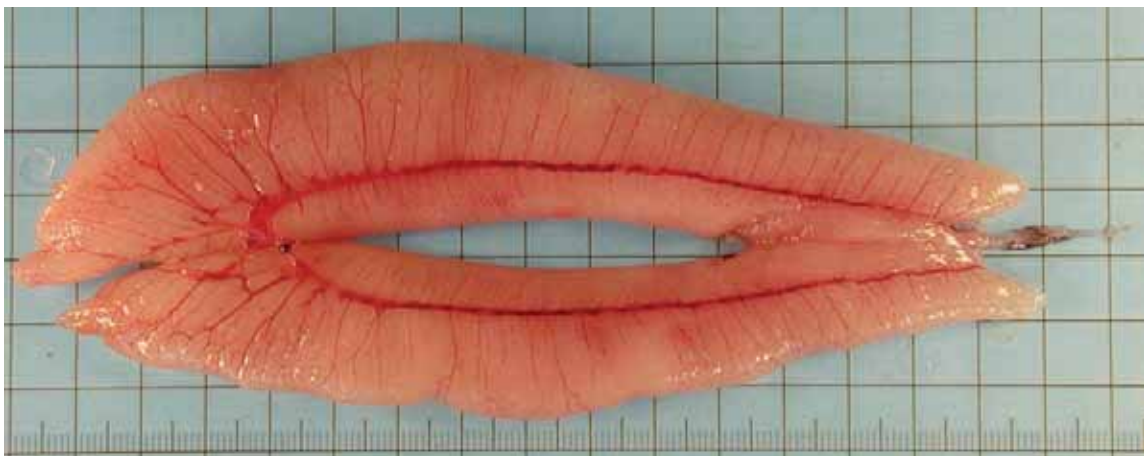
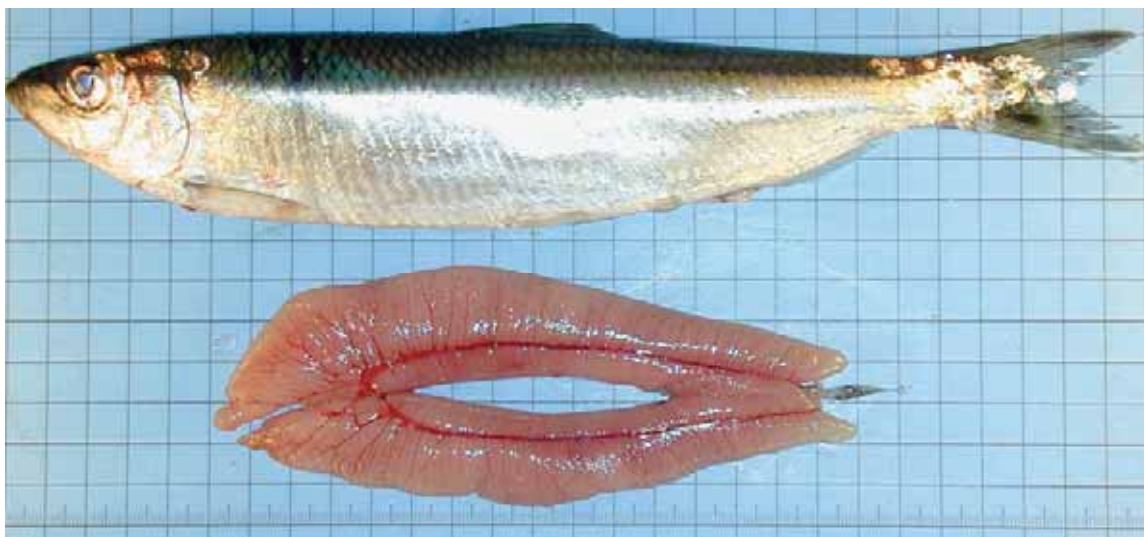
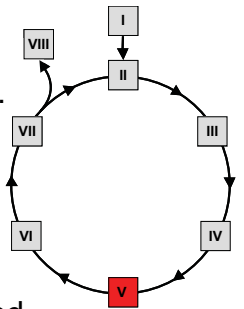
L<sub>T</sub>: 28.5 cm  
M<sub>B</sub>: 193.9 g  
M<sub>G</sub>: 47.9 g  
GSI: 32.8  
M: May 2006  
ID: 080506s/5



### V. Spawning prepared



This example illustrates the variation in colour and transparency within stage V. Specimens are close to spawning, but the most important traits of this stage are: 1) Large volume of the ovary 2) Transparency of the eggs 3) No eggs released with slight pressure to the abdomen.



#### Enlargement

Oviducts are filled with eggs but none are present in the most posterior part. The individual egg appears clear whitish translucent to transparent.

#### Specimen data

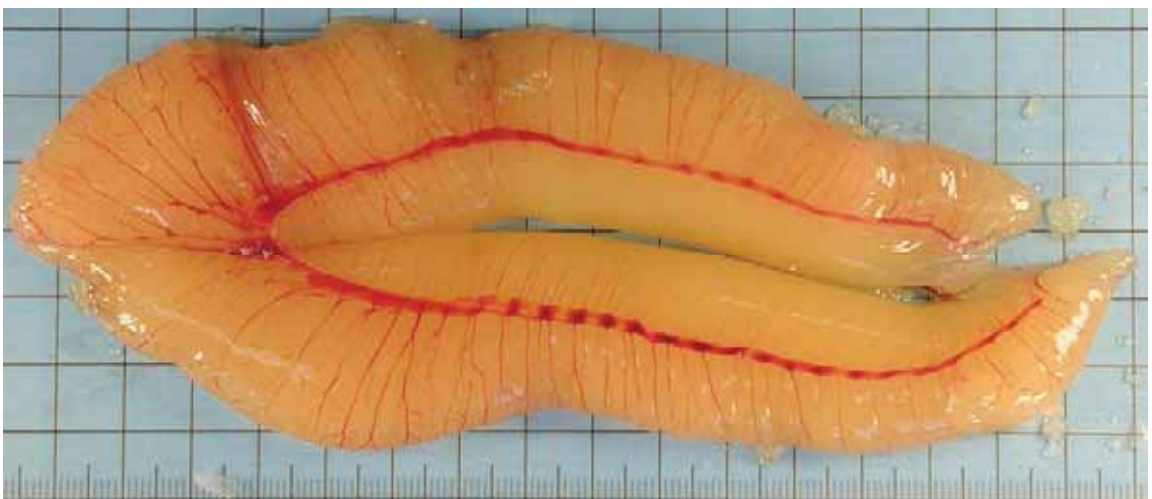
L<sub>T</sub>: 27.5 cm  
M<sub>B</sub>: 167.3 g  
M<sub>G</sub>: 47.3 g  
GSI: 39.4  
M: May 2006  
ID: 080506s/17

## VI. Spawning active ♀

### VI. Spawning active



Ovaries still occupy the full length of the body cavity, but the ventral part gradually shrinks during spawning; colour is bright translucent yellow; eggs are released with slight pressure to abdomen or flow freely; ovary consistency is soft and surface smooth.



#### Enlargement

The eggs have increased in size due to hydration and ovulated eggs appear in the oviduct. The colour of the eggs is transparent clear yellow-whitish.

#### Specimen data

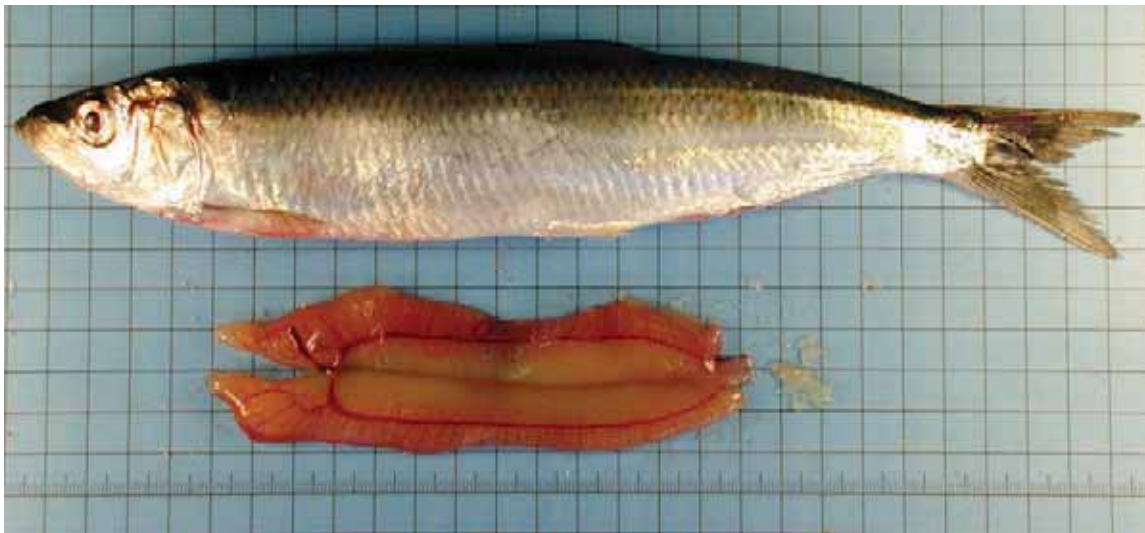
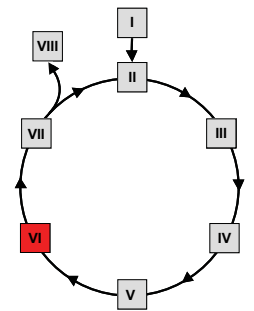
L<sub>T</sub>: 30 cm  
M<sub>B</sub>: 141.7 g  
M<sub>G</sub>: 64.2 g  
GSI: 42.7  
M: May 2006  
ID: 080506s/40



### VI. Spawning active



In this example spawning has progressed further. The ventral half of the ovary becomes increasingly striated and bloodshot, as the eggs are gradually ovulated and released into the dorsally located oviduct.



#### Enlargement

Hydrated eggs are present in the most distal part of the oviduct, and may flow freely from this. A whitish-pink pattern appears in the ventral part of the ovary as the eggs have been ovulated and the follicle remains accumulate.

#### Specimen data

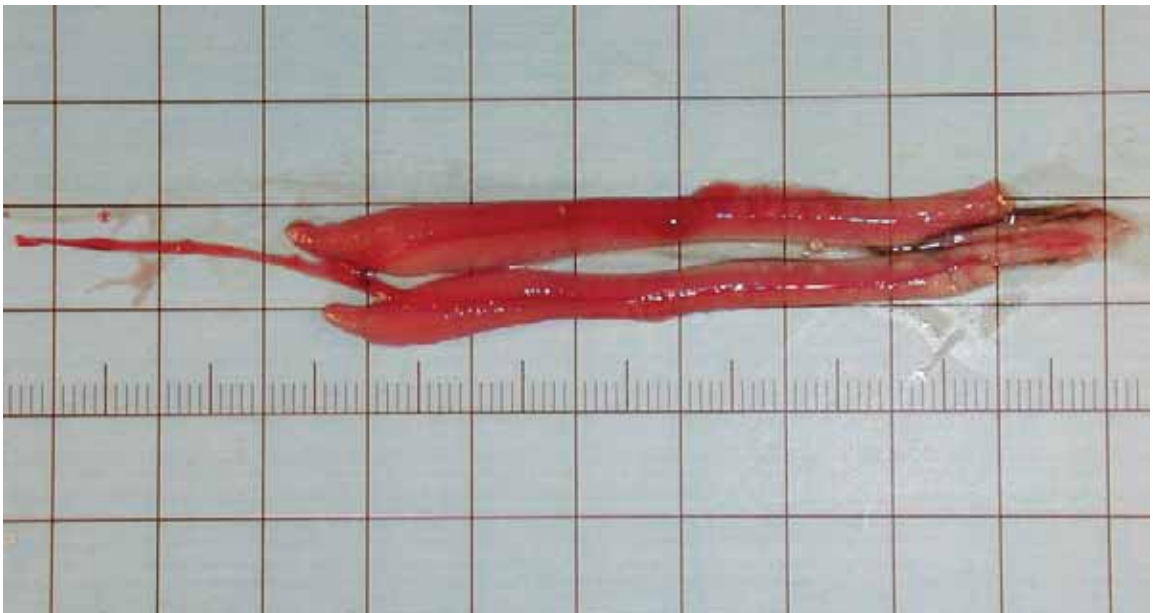
L<sub>T</sub>: 27 cm  
M<sub>B</sub>: 135.3 g  
M<sub>G</sub>: 16.9 g  
GSI: 14.3  
M: May 2006  
ID: 080506s/47

## VII. Spent - regeneration ♀

### VII. Spent - regeneration



Ovaries occupy from less than 1/2 to more than 2/3 the length of the body cavity; 5-8 mm wide, but narrower towards the posterior; translucent orange to red; some with clear transparent posterior and/or anterior ends; flaccid and shrunken; main artery prominent.



#### Enlargement

The oviduct is enlarged distally as a sign of recent spawning and in some cases transparent areas are seen towards the posterior or anterior.

#### Specimen data

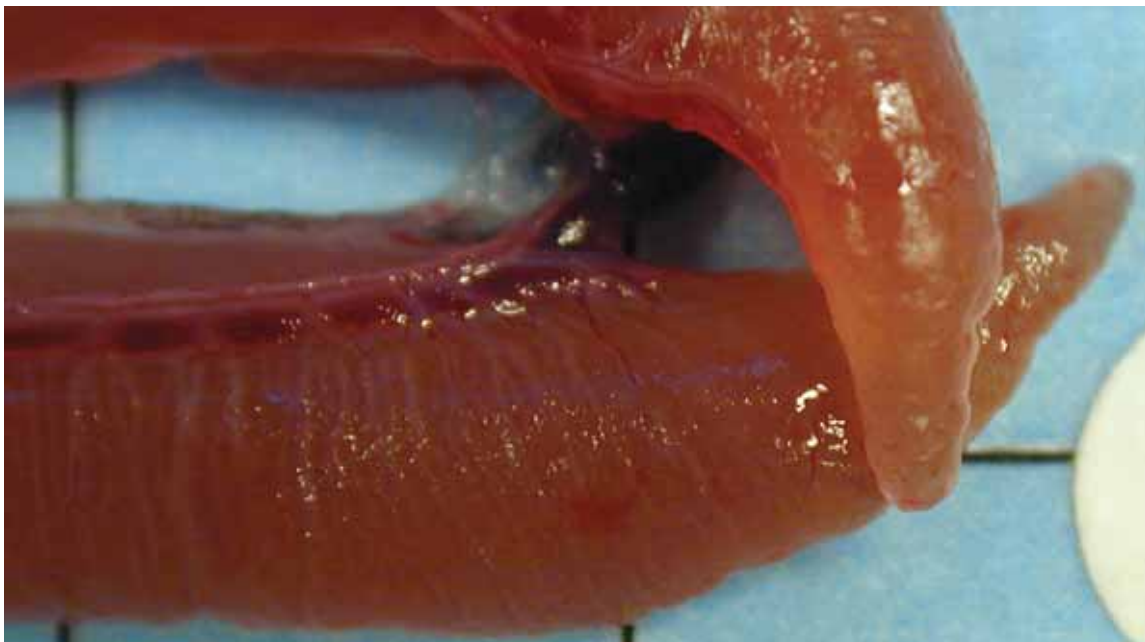
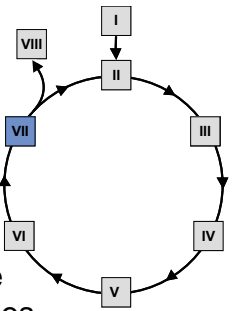
L<sub>T</sub>: 23.5 cm  
M<sub>B</sub>: 68.9 g  
M<sub>G</sub>: 0.3 g  
GSI: 0.4  
M: May 2006  
ID: 080506s/53



### VII. Spent - regeneration



This example illustrates the variation in size, shape and colour within stage VII. As the ovary wall contracts the transverse grooves appear again and the ovary becomes more firm. As in stage II repeat spawners the transverse grooves sometimes appear grey.



#### Enlargement

Enlarged oviduct (left).

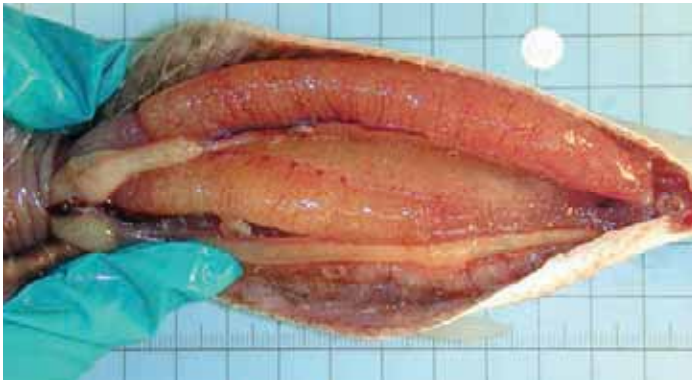
**TIP:** Stage VII can often only be distinguished from stage II repeat spawners by checking the absence of ca oocytes microscopically. In stage VII only immature oocytes are present as in stage I (see p. 14-17).

#### Specimen data

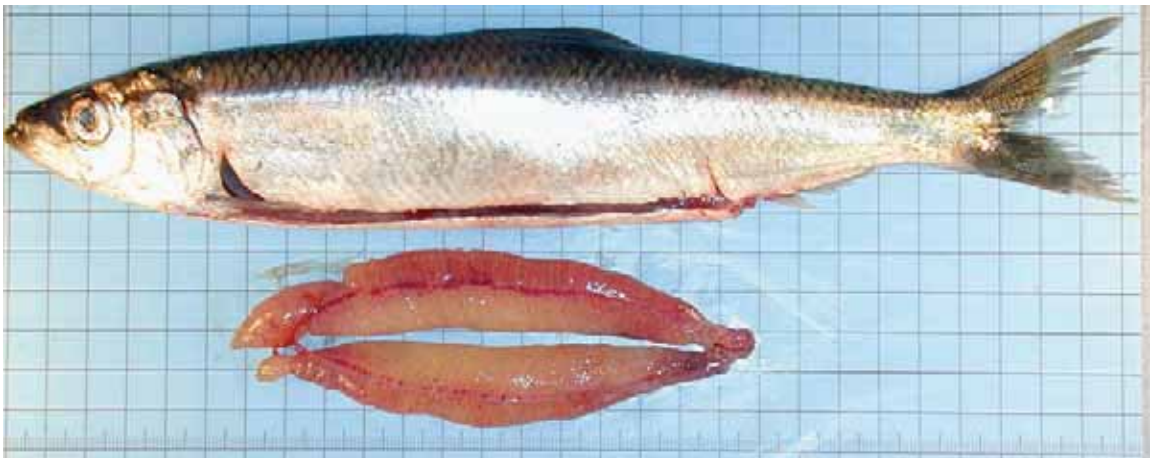
L<sub>T</sub>: 26 cm  
M<sub>B</sub>: 108 g  
M<sub>G</sub>: 1.6 g  
GSI: 1.5  
M: November 2004  
ID: 010405s/61

## VIII. Abnormal ♀

### VIII. Abnormal



Ovaries are subject to a variety of developmental abnormalities and only two examples are shown here. In this example the oviduct has fused together near the opening and the oocytes are trapped inside the ovary. Many atretic oocytes are visible.



#### Enlargement

The oviduct has fused near the opening. The trapped oocytes are atretic, i.e. they are being reabsorbed, and appear more opaque white than normal mature hydrated eggs.

#### Specimen data

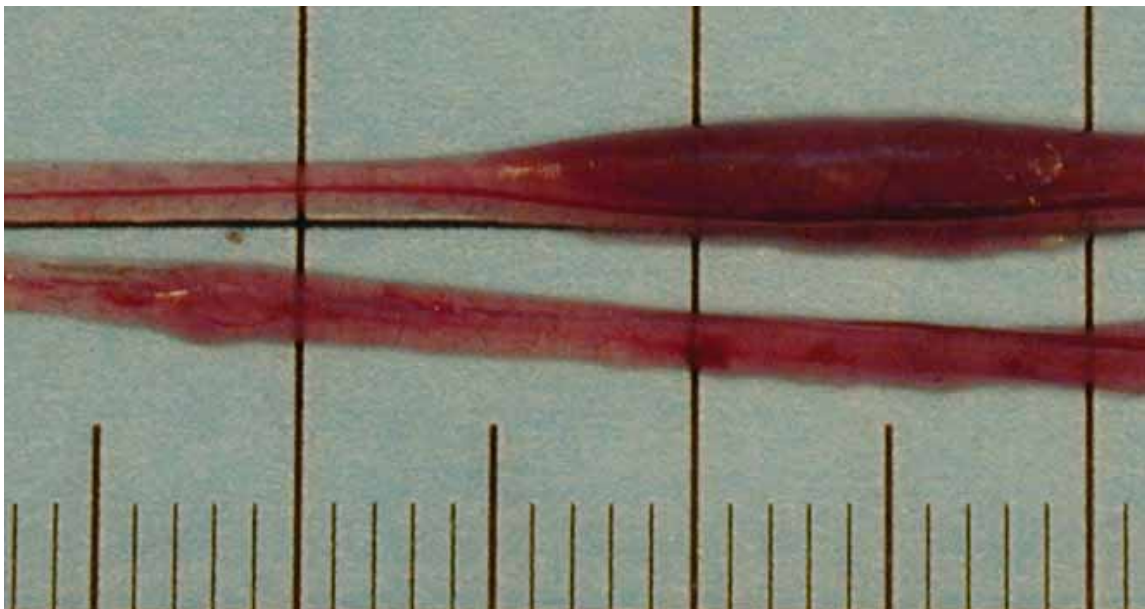
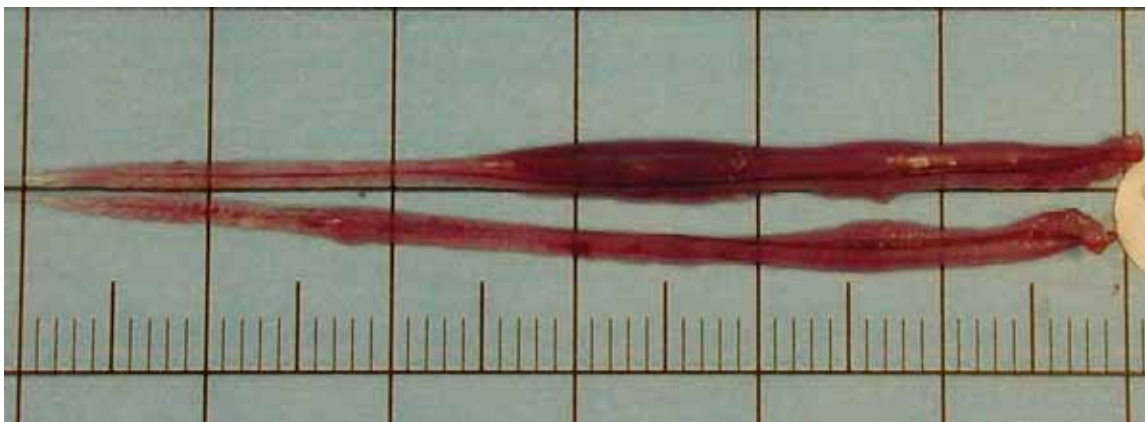
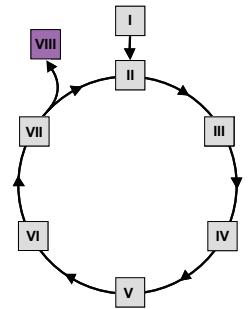
L<sub>T</sub>: 28 cm  
GSI: 20.4  
M<sub>B</sub>: 146.7 g  
M: May 2006  
M<sub>G</sub>: 30 g  
ID: 080506s/11



## VIII. Abnormal



This example shows a bisexual individual. At first glance it looks like a normal ovary. But a closer look reveals a female anterior half and a male posterior half.



### Enlargement

The anterior half of the gonad contains mainly oocytes and looks similar to a developing ovary. But histological sections reveal that in fact both oocytes and spermatocytes are present.

### Specimen data

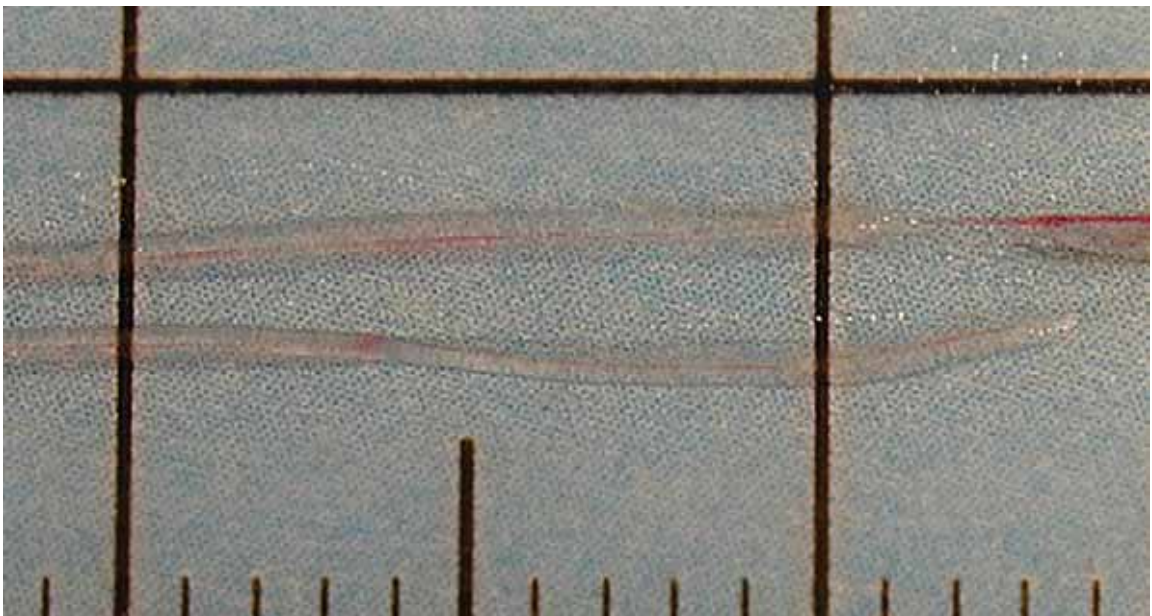
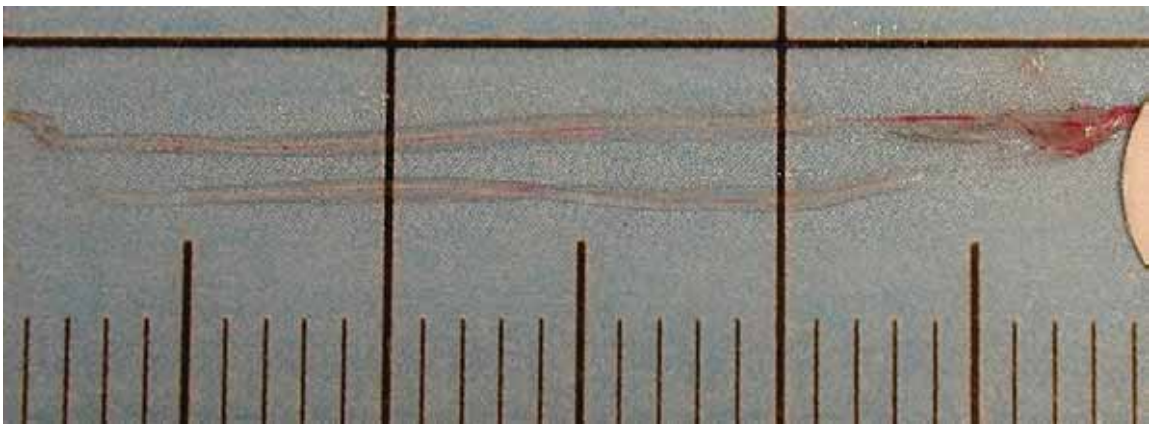
L<sub>T</sub>: 26 cm  
M<sub>B</sub>: 116.4 g  
M<sub>G</sub>: 0.4 g  
GSI: 0.3  
M: November 2004  
ID: 010405s/9

## I. Juvenile ♂

### I. Juvenile



Testes emerge as thin, flat paired organs occupying around 1/3 – 1/2 of the length of the body cavity; a few mm wide; transparent to whitish translucent; distinguishable from ovaries by the lanceolate shape; the sperm duct is not visible to the naked eye.



#### Enlargement

The surface of the immature testes is smooth; vascularisation is limited and the main artery is relatively narrow. There are no signs of development or of previous spawning.

#### Specimen data

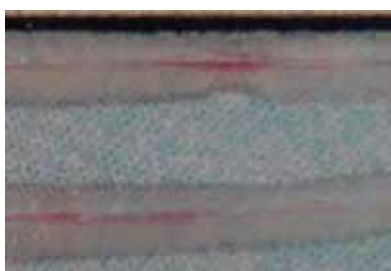
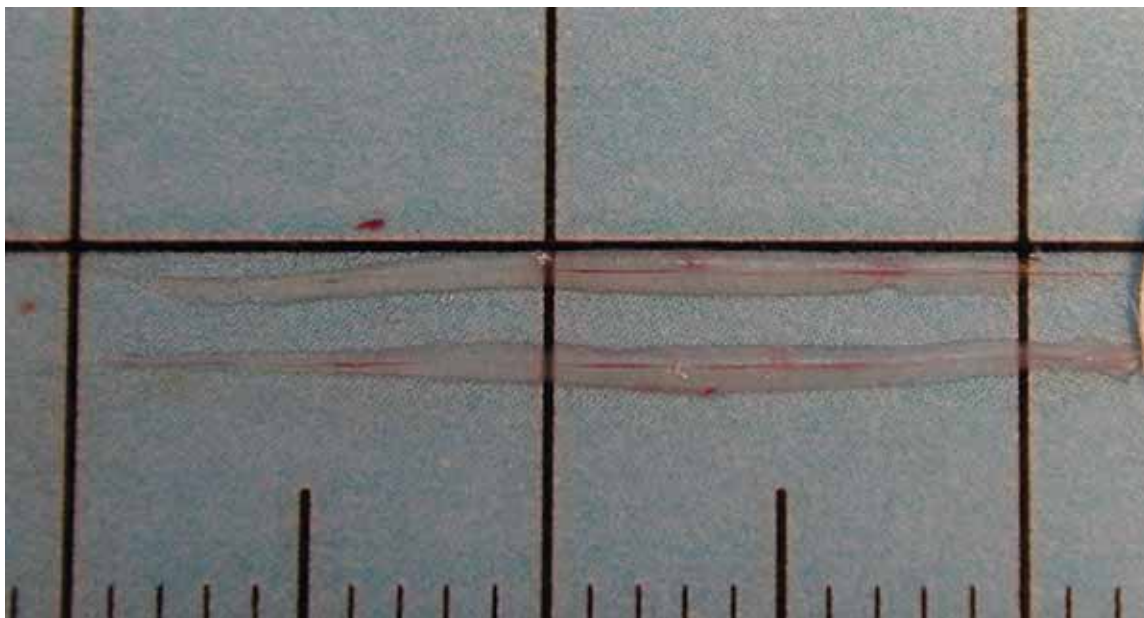
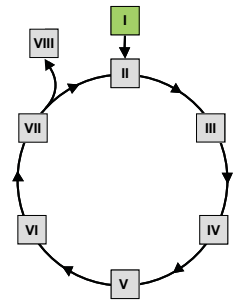
L<sub>T</sub>: 12 cm  
M<sub>B</sub>: 12.1 g  
M<sub>G</sub>: < 0.1 g  
GSI: ~ 0  
M: November 2004  
ID: 010405s/21



## I. Juvenile



This example illustrates the variation in colour within stage I, which ranges from transparent to whitish translucent.



### Enlargement

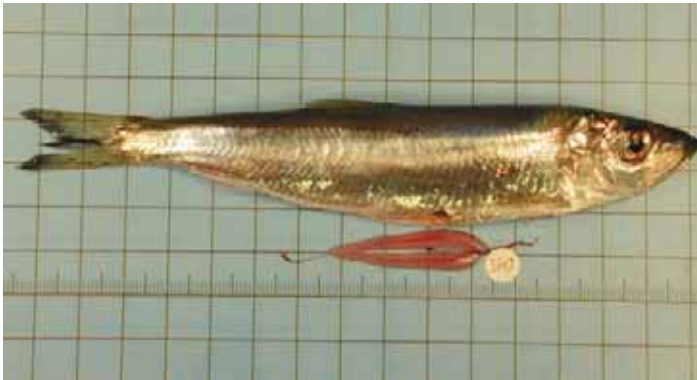
**TIP:** It can be tricky to distinguish immature males from females. But if the gonad is flat, knife-shaped and has a smooth surface it is a male!

### Specimen data

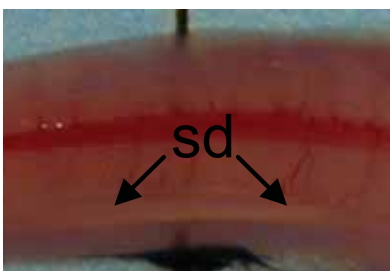
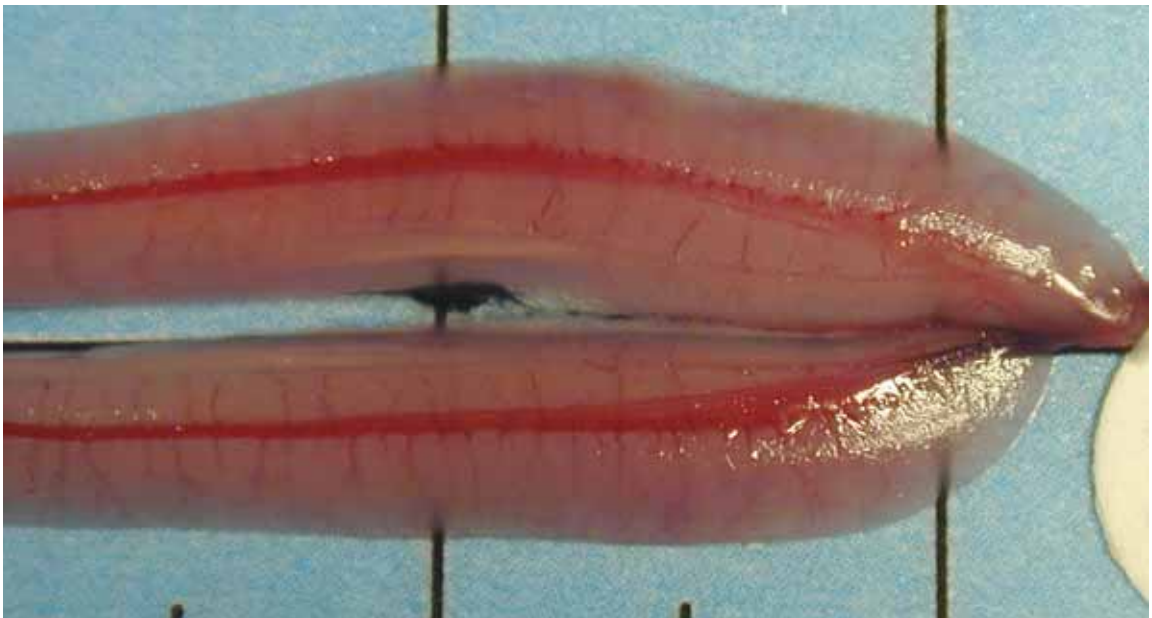
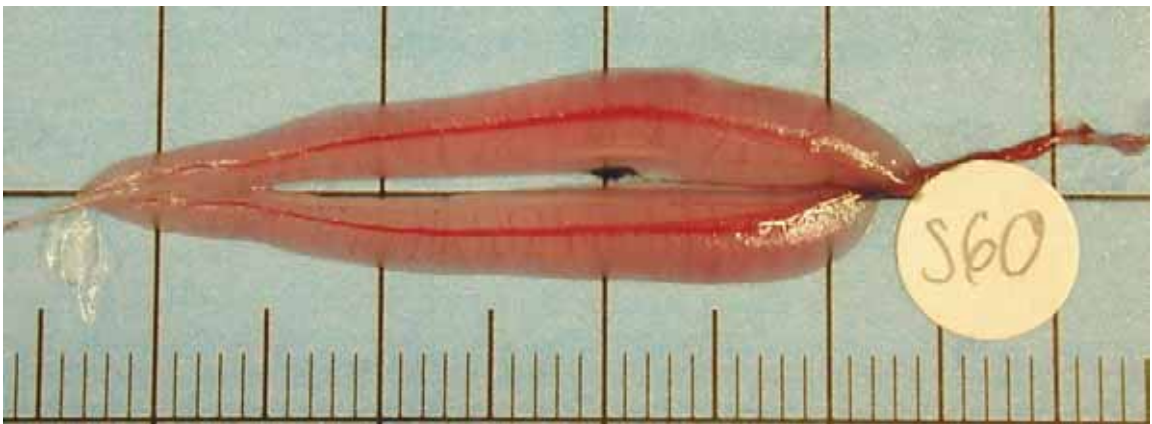
$L_T$ : 13 cm  
 $M_B$ : 13.7 g  
 $M_G$ : < 0.1 g  
 $GSI$ : ~ 0  
 M: November 2004  
 ID: 010405s/26

## II. Early maturation ♂

### II. Early maturation – first time spawner



Testes occupy  $\frac{1}{2}$  the length of the body cavity; around 5 mm wide, but narrower towards the posterior; translucent whitish-pink; edges remain transparent; the sperm duct is visible early in the stage as a tubule dorsally.



#### Enlargement

Edges remain transparent.

**TIP: Stage II first time spawners are distinguished from stage I by**

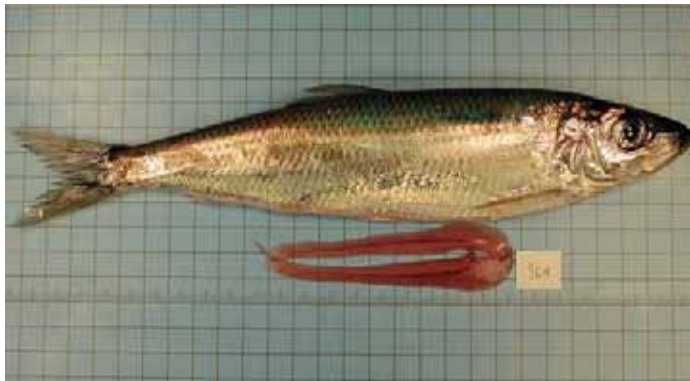
- 1) Increased vascularization**
- 2) Increased opaqueness**
- 3) Easily detectable sperm duct (sd)**

#### Specimen data

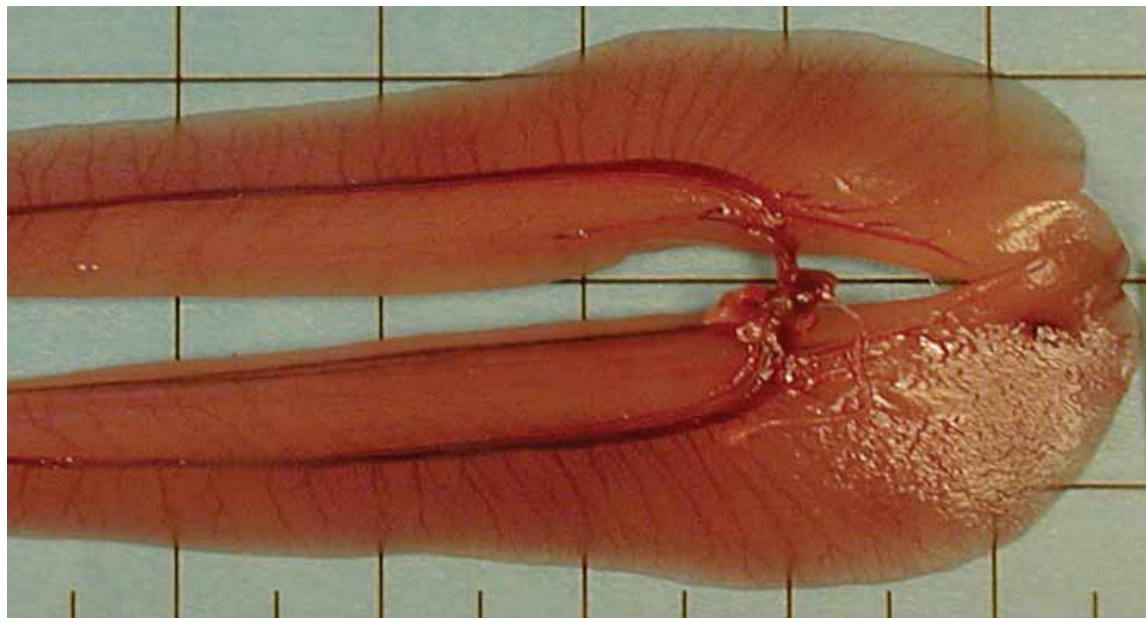
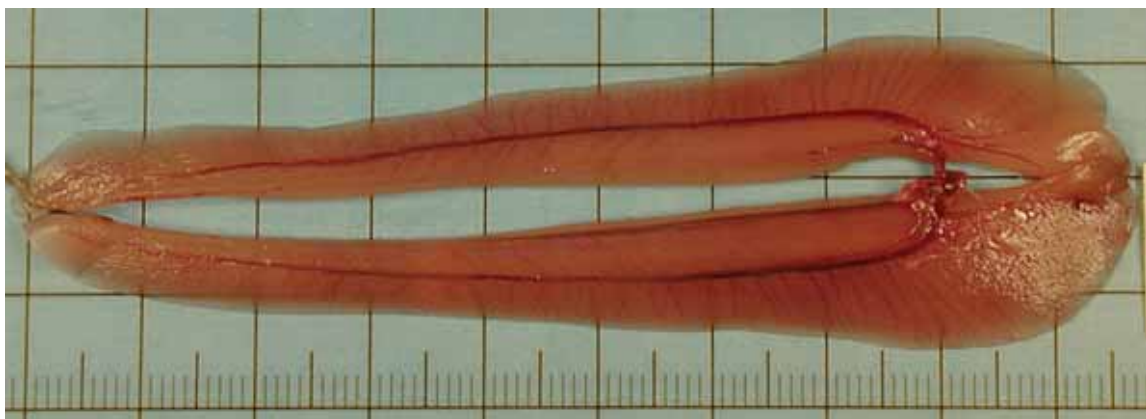
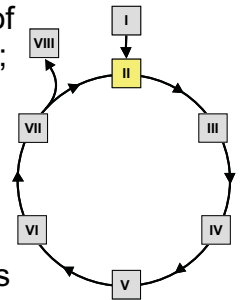
L<sub>T</sub>: 15 cm  
M<sub>B</sub>: 22.2 g  
M<sub>G</sub>: 0.3 g  
GSI: 1.4  
M: November 2004  
ID: 010405s/99



### II. Early maturation – repeat spawner



Testes occupy 2/3 or more of the length of the body cavity; 5-15 mm wide, narrower towards the posterior; translucent pink to orange-red; edges transparent; shape clearly lanceolate; sperm duct is visible and has a tubule running almost the entire length dorsally.



#### Enlargement

Edges are transparent. **TIP:** It can be tricky to distinguish stage VII from stage II repeat spawners, but in stage II the longitudinal valves created by the contraction of the testes (see stage VII) during and after spawning have disappeared.

#### *Specimen data*

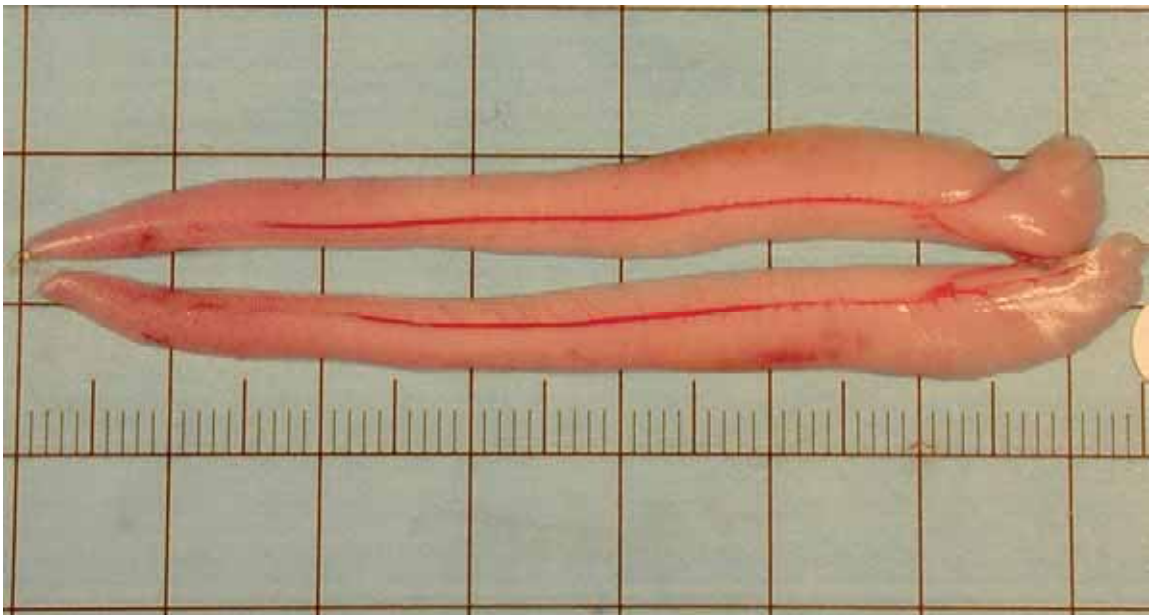
$L_T$ : 27 cm  
 $M_B$ : 138.8 g  
 $M_G$ : 3.4 g  
 $GSI$ : 2.4  
 M: November 2004  
 ID: 010405s/58

### III. Mid maturation ♂

#### III. Mid maturation



Testes occupy between 2/3 and the entire length of the body cavity; 5-15 mm wide and narrower towards the posterior end; colour varies from pale orange-pink to pale whitish-pink; increasingly opaque but transparent edges persist until late in stage.



#### Enlargement

Edges are still transparent in early stage III testes.

**TIP: Stage III is distinguished from stage II by the more opaque whitish colouration and thicker appearance due to the increased production of spermatozoa.**

#### Specimen data

L<sub>T</sub>: 16 cm  
M<sub>B</sub>: 26.1 g  
M<sub>G</sub>: 1.3 g  
GSI: 5  
M: November 2004  
ID: 010405s/63

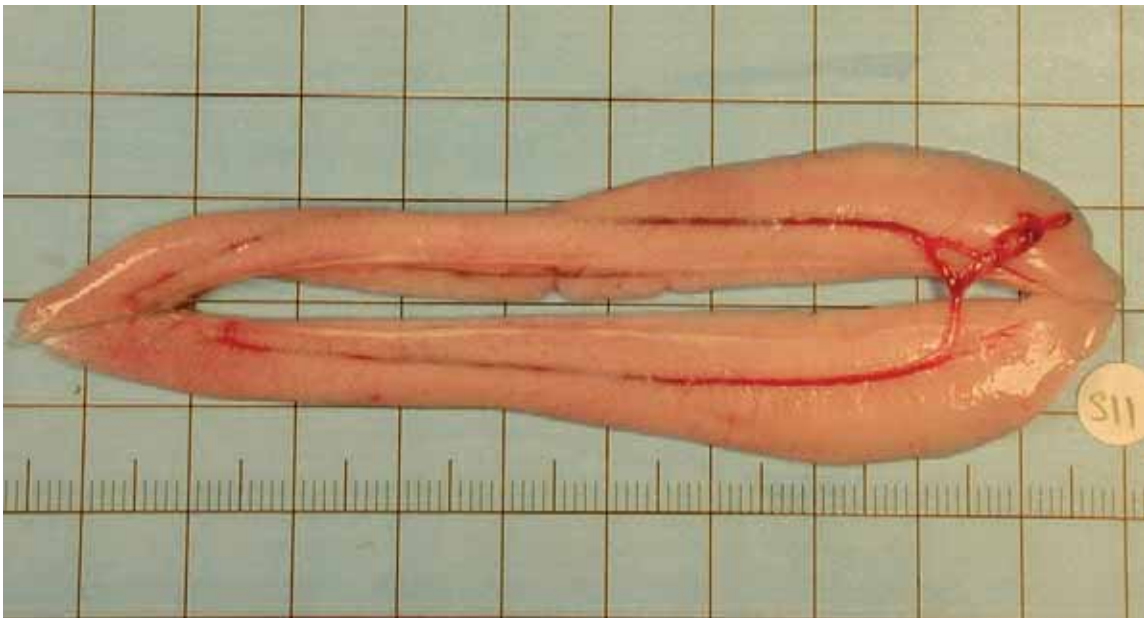
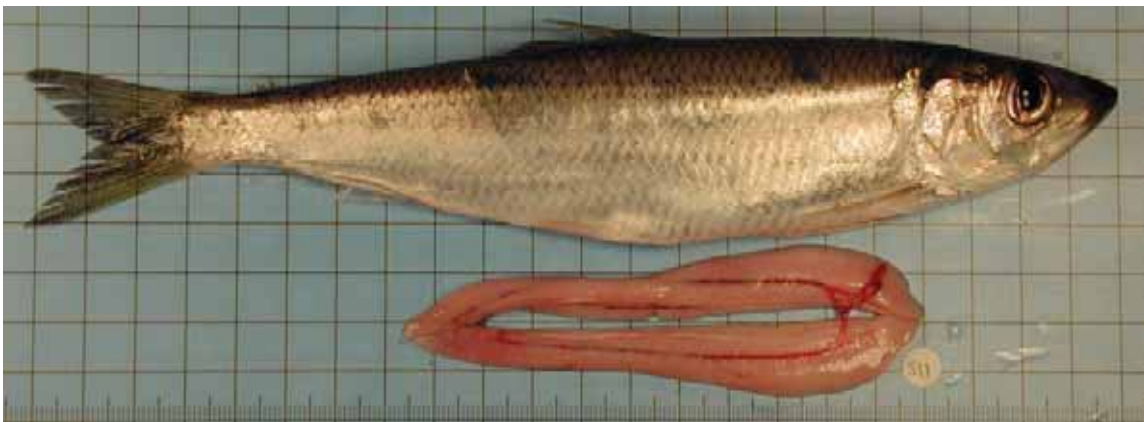
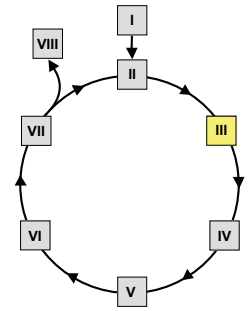


### III. Mid maturation ♂

#### III. Mid maturation



This example indicates variation in colour and size within stage III. At this and later stages of maturation it is not possible to distinguish between first time and repeat spawners with the naked eye.



#### Enlargement

By the end of stage III the edges appear opaque as does the rest of the gonad. The testes become less firm during maturation and ruptures more easily.

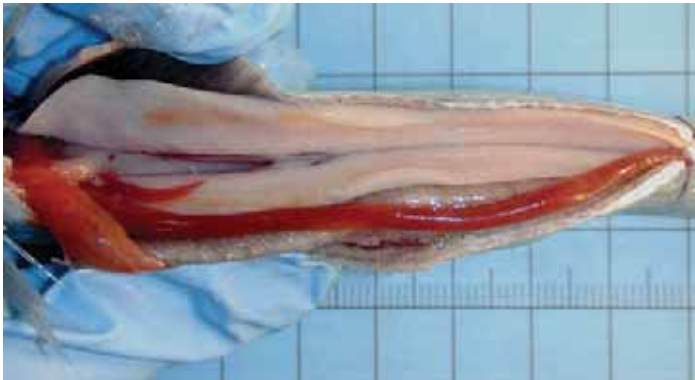
#### Specimen data

L<sub>T</sub>: 21 cm  
 M<sub>B</sub>: 71.1 g  
 M<sub>G</sub>: 6.1 g  
 GSI: 8.6  
 M: November 2004  
 ID: 010405s/50

III

## IV. Final maturation ♂

### IV. Final maturation



The testes occupy the entire length and almost the entire volume of the body cavity; 15 - 25 mm at the widest point and narrower towards the posterior end; colour is opaque and very pale whitish-pink with white tints.



#### Enlargement

Whitish tints are visible due to increased proportion of spermatozoa in the seminiferous tubules.

**TIP: The complete opaqueness and the dimensions of the testes are the most important traits distinguishing stage IV from stage III.**

#### Specimen data

L<sub>T</sub>: 19.1 cm  
M<sub>B</sub>: 44 g  
M<sub>G</sub>: 5.5 g  
GSI: 12.5  
M: March 2006  
ID: 300306s/30

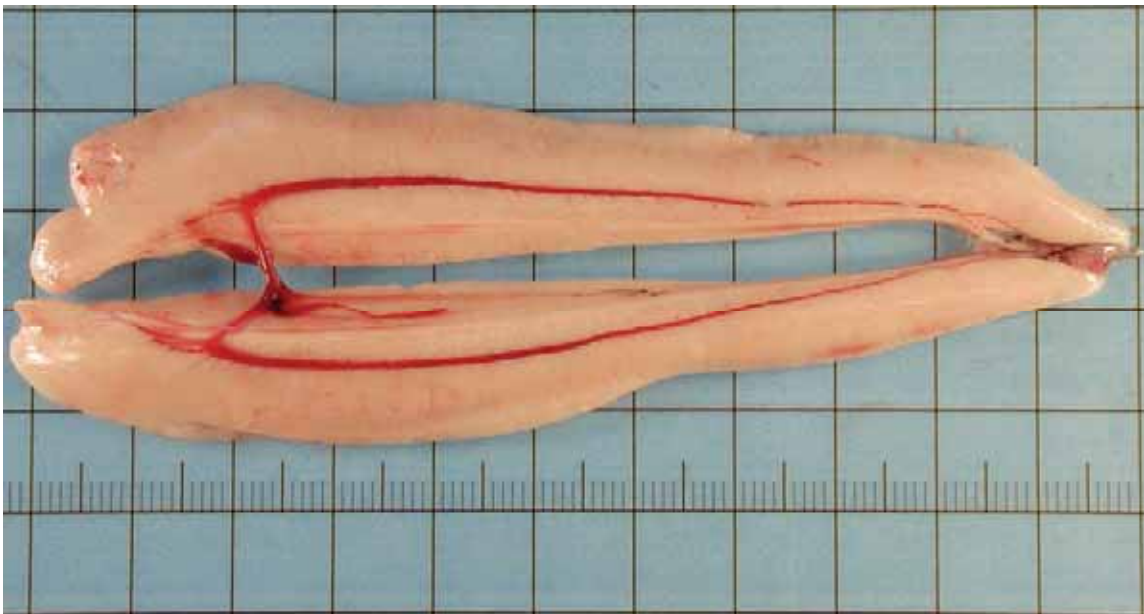
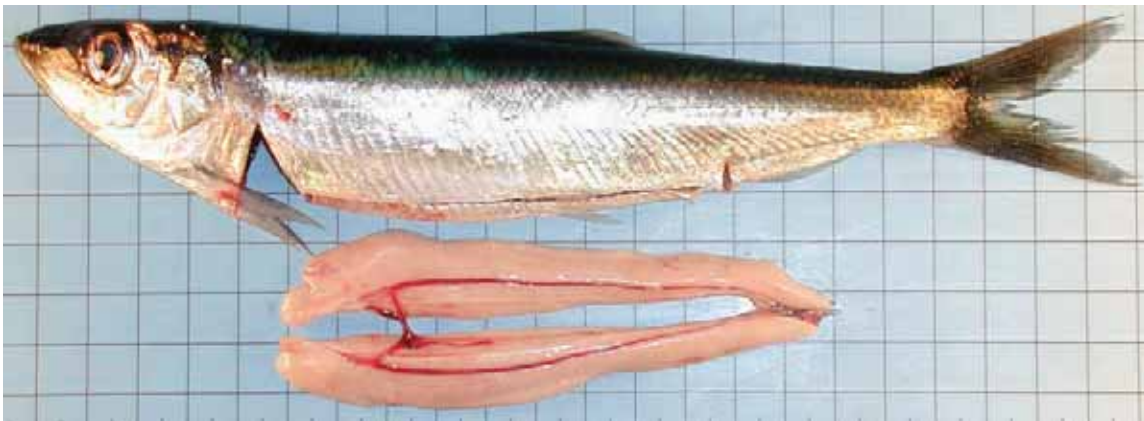
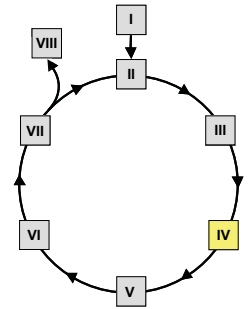


## IV. Final maturation ♂

### IV. Final maturation



This example illustrates variation in size and shape within stage IV. Generally the colour varies little between different specimens in this stage.



#### Enlargement

The two lobes of the testes become less flaccid and more thick and swollen during the maturation process.

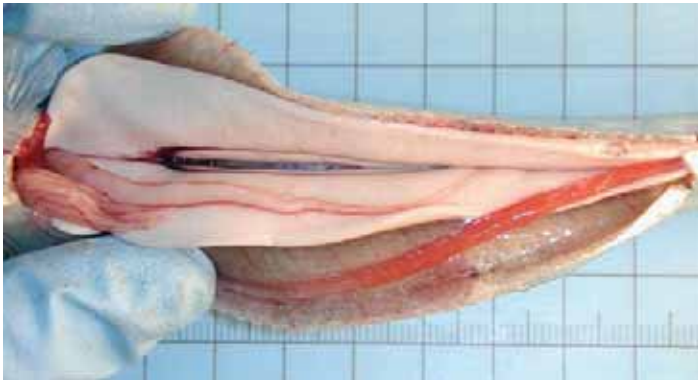
#### *Specimen data*

*L<sub>T</sub>*: 22.4 cm  
*M<sub>B</sub>*: 64 g  
*M<sub>G</sub>*: 9.9 g  
*GSI*: 15.5  
*M*: March 2006  
*ID*: 300306s/46

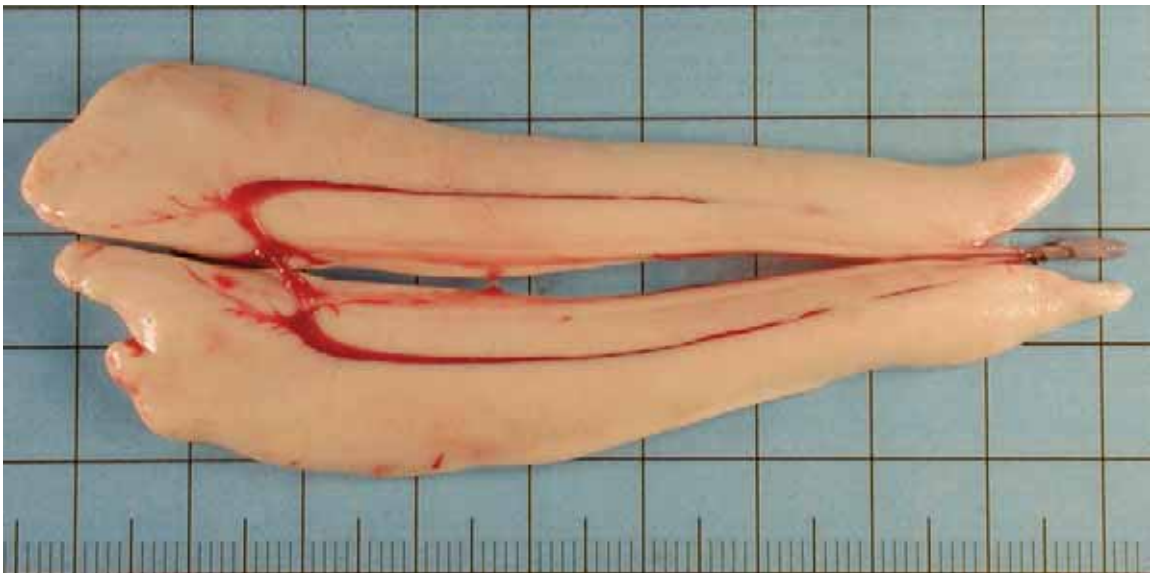
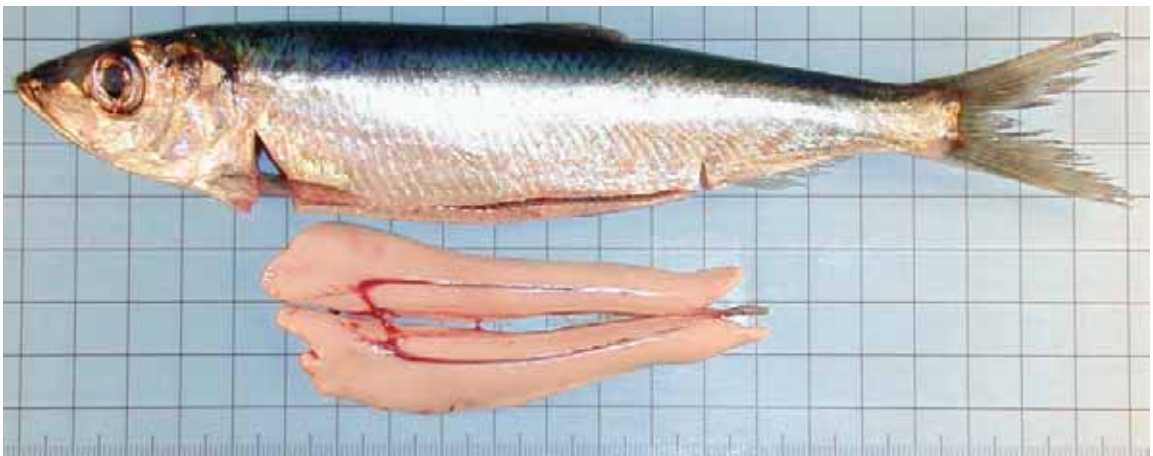
IV

## V. Spawning prepared ♂

### V. Spawning prepared



Testes occupy the entire volume of the body cavity; 15 to more than 30 mm at the widest point and narrower towards the posterior; colour opaque, pale pinkish-white to milky-white; a small amount of sperm may be released with pressure, but sperm does not flow freely.



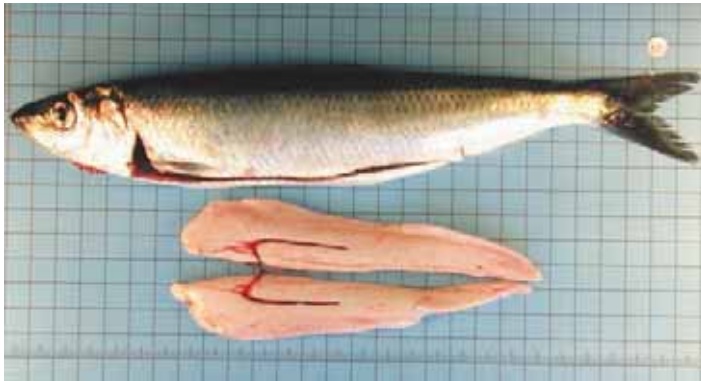
#### Enlargement

Tip: Testes are close to spawning but the most important traits distinguishing stage V are 1) Testes now occupy the entire volume of the body cavity 2) Sperm duct is distended throughout entire length 3) Sperm does not run freely.

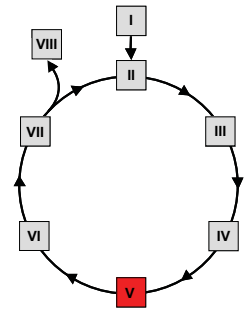
#### Specimen data

L<sub>T</sub>: 20.8 cm  
M<sub>B</sub>: 58 g  
M<sub>G</sub>: 8.9 g  
GSI: 15.3  
M: March 2006  
ID: 300306s/43

### V. Spawning prepared



This example shows the variation in colour and shape within stage V. Generally the colour varies only little between different specimens in this stage, but red tints may be visible in some tissues.



#### Enlargement

The colouration is more evenly pinkish-white or milky-white since spermatozoa occupy most of the testes volume.

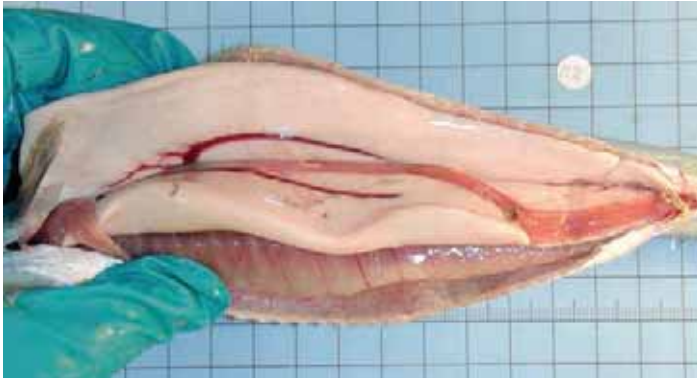
#### Specimen data

L<sub>T</sub>: 27.5 cm  
M<sub>B</sub>: 147.8 g  
M<sub>G</sub>: 31.5 g  
GSI: 21.3  
M: May 2006  
ID: 080506s/1

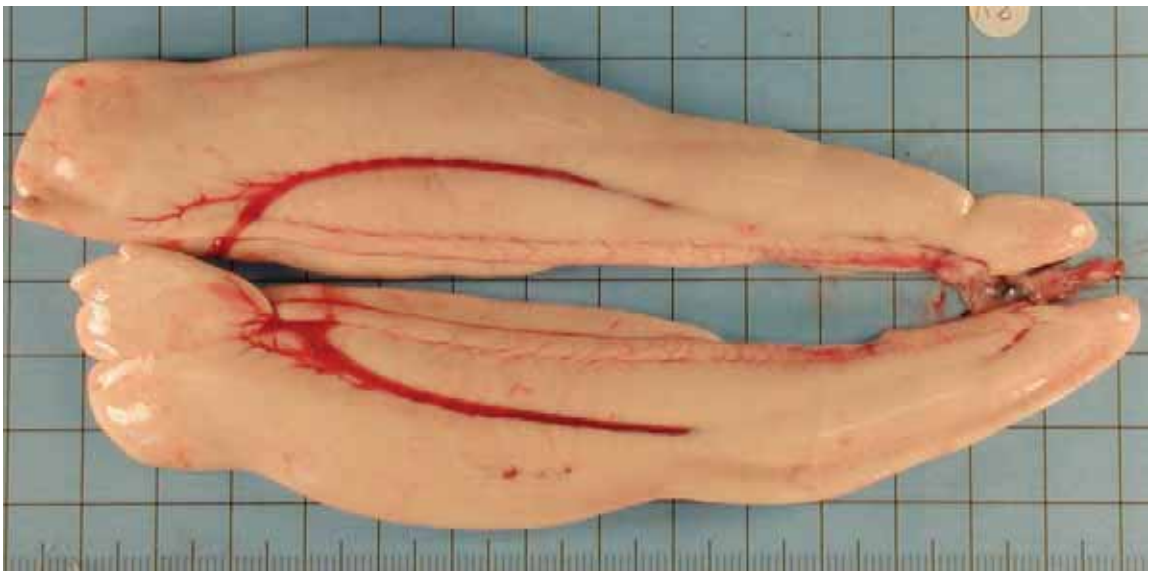


## VI. Spawning active ♂

### VI. Spawning active



Testes still occupy the full length of the body cavity, but width gradually diminishes during spawning; colour opaque, pale pinkish-white to milky-white; testes consistency is soft and surface smooth; sperm flows freely.



#### Enlargement

The sperm duct is extremely distended and the testes have a smoother appearance.

**But most importantly the sperm now runs freely from the sperm duct!**

#### Specimen data

L<sub>T</sub>: 28 cm

M<sub>B</sub>: 183 g

M<sub>G</sub>: 46.2 g

GSI: 25.2

M: May 2006

ID: 080506s/8

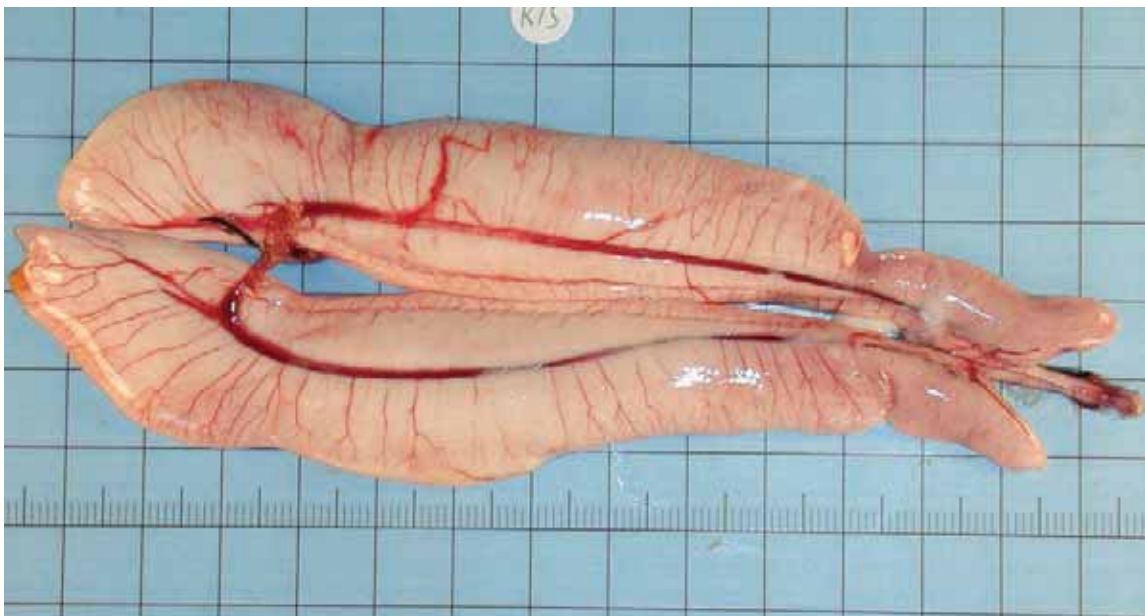
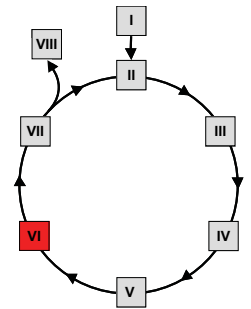


## VI. Spawning active ♂

### VI. Spawning active



This example illustrates variation within stage VI. As spawning progresses the testes become increasingly striated and bloodshot, as sperm is released into the dorsally located sperm duct.



#### Enlargement

The testes become bloodshot and can display an almost purple tint when sperm has been released by contractions of the testicular wall.

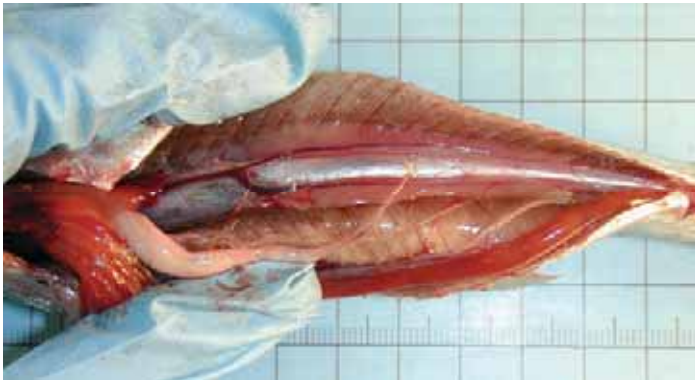
#### Specimen data

L<sub>T</sub>: 26.5 cm  
M<sub>B</sub>: 150.4 g  
M<sub>G</sub>: 22.4 g  
GSI: 14,9  
M: May 2006  
ID: 080506s/15

VI

## VII. Spent – regeneration ♂

### VII. Spent - regeneration



Testes occupy from around 1/2 to 2/3 the length of the body cavity; 3-8 mm wide, but narrower towards the posterior; translucent pale pink, orange or reddish-orange; transparent along edges; flat and shrunken; grooves running longitudinally; main artery prominent.



#### Enlargement

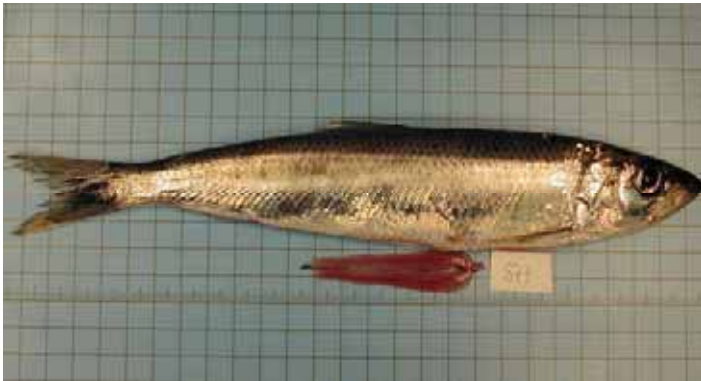
TIP: It can be tricky to distinguish stage VII from stage II repeat spawners. But in stage VII longitudinal grooves from the contraction of the testicular wall are visible. Early in stage VII the sperm duct may also be enlarged towards the posterior (picture above).

#### Specimen data

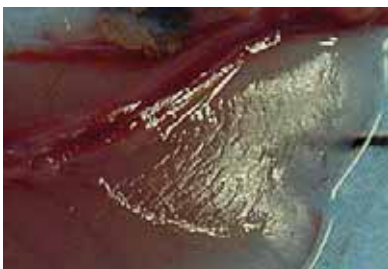
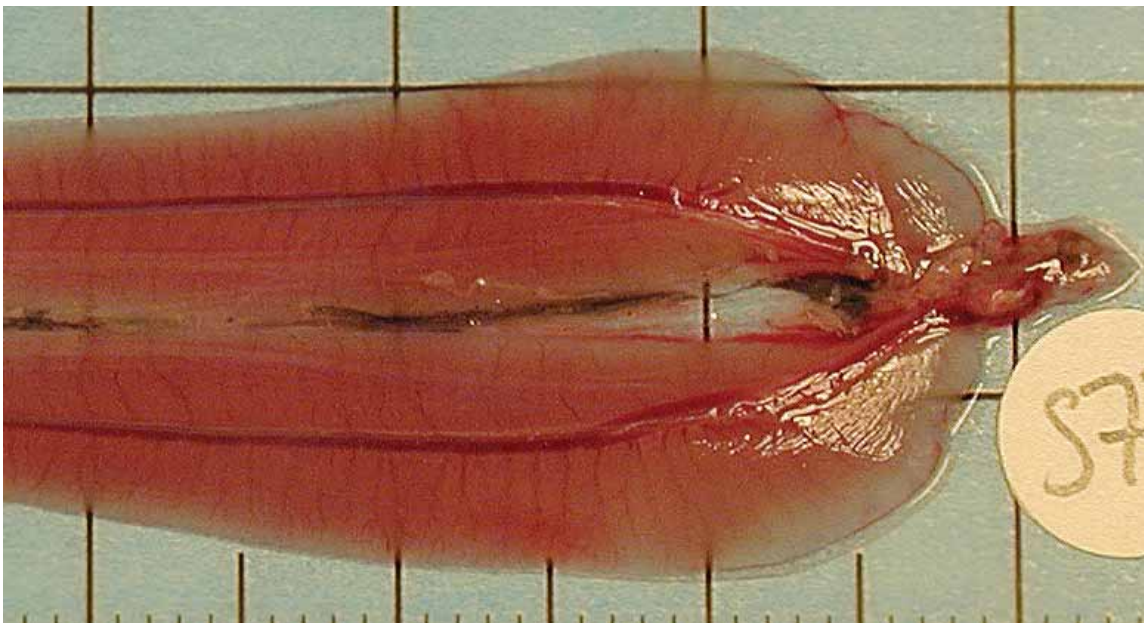
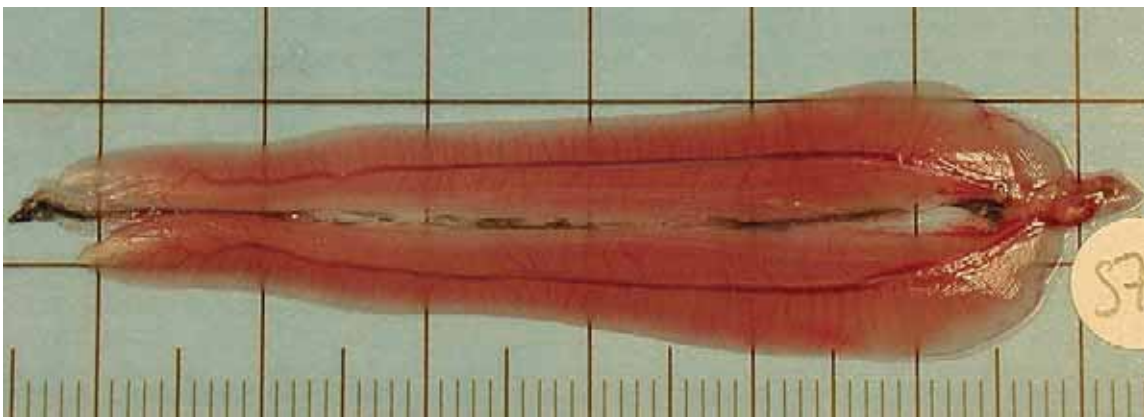
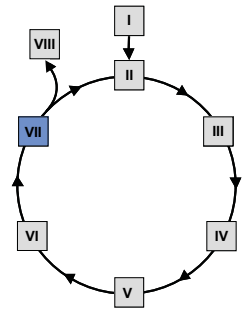
L<sub>T</sub>: 22.7 cm  
M<sub>B</sub>: 66 g  
M<sub>G</sub>: 0.6 g  
GSI: 0.9  
M: March 2006  
ID: 300306s/24



### VII. Spent - regeneration



This example illustrates the variation in size, shape and colour within stage VII. By the end of stage VII spawning marks are less evident and in some cases it is only possible to distinguish stage VII and stage II repeat spawners histologically.



#### Enlargement

By the end of stage VII the longitudinal grooves become less pronounced as the testes gradually begin to recover and start a new production of germ cells. The main artery is prominent and testes are transparent along the edges.

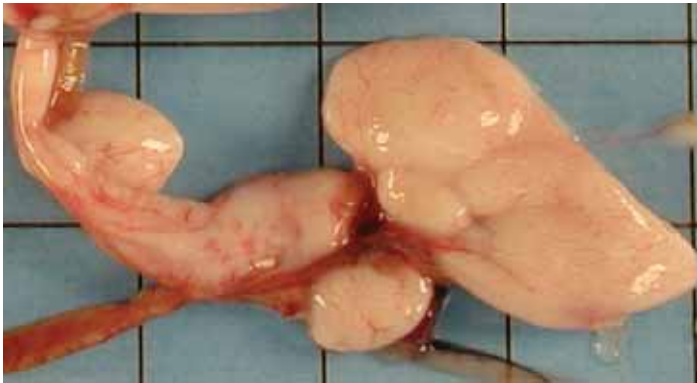
#### Specimen data

L<sub>T</sub>: 25 cm  
M<sub>B</sub>: 102.3 g  
M<sub>G</sub>: 1.1 g  
GSI: 1.1  
M: November 2004  
ID: 010405s/110

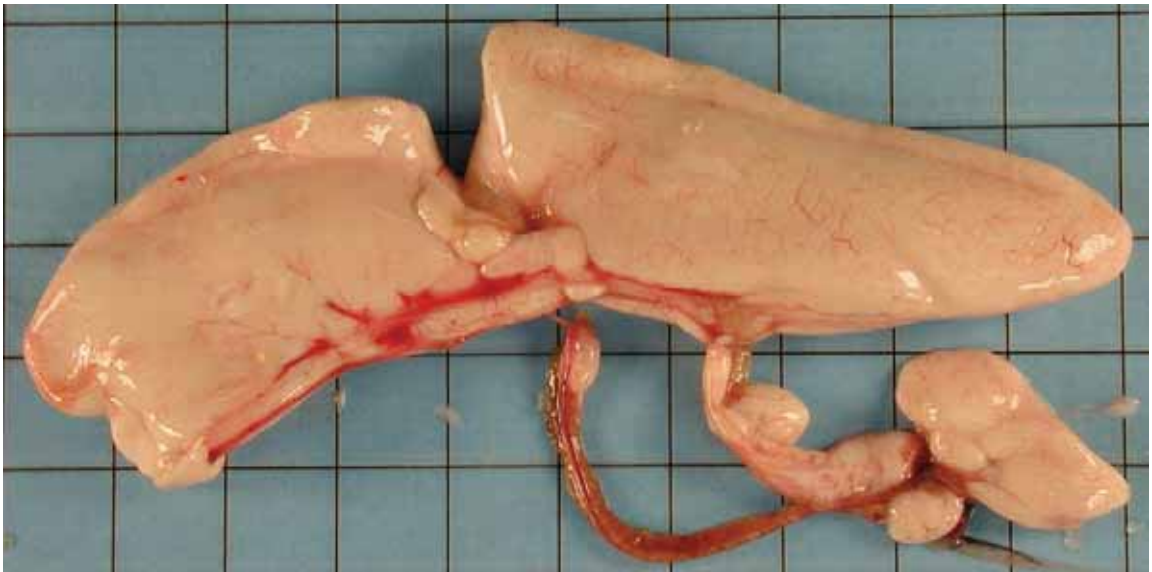


## VIII. Abnormal ♂

### VIII. Abnormal



Testes are subject to a variety of developmental abnormalities and two examples are shown here. One of the more common abnormalities is the lack of development of a part of the testes. In the example provided only one lobe had developed.



#### Enlargement

The undeveloped lobe. Although abnormalities occur relatively infrequently it is important to recognize such phenomena as potential indicators of ecosystem changes such as increased amounts of pollutants.

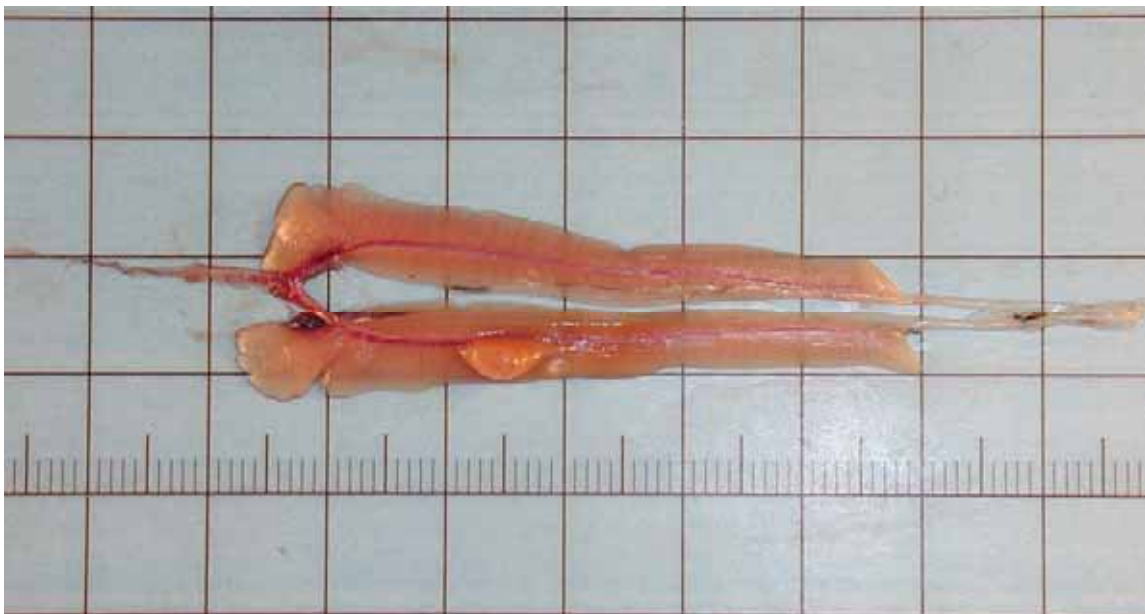
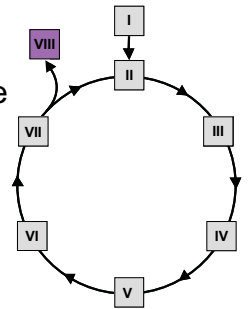
#### Specimen data

M<sub>B</sub>: 94.7 g  
M<sub>G</sub>: 13.9 g  
L<sub>T</sub>: 23.5 cm  
GSI: 14.7  
M: May 2006  
ID: 080506s/63

## VIII. Abnormal



This example shows a bisexual individual. At first glance the gonad looks like normal testes, except for the "knob" of tissue. This actually contains oocytes! The remaining testes however appear functional and have completed spawning.



### Enlargement

The abnormal extrusion or "knob" containing oocytes indicates reproductive disturbance.

### *Specimen data*

*M<sub>B</sub>*: 76 g  
*M<sub>G</sub>*: 0.8 g  
*L<sub>T</sub>*: 23.5 cm  
*GSI*: 1.05  
*M*: March 2006  
*ID*: 300306s/23